DIFFERENTIAL EFFECTS OF CONTROLLABLE AND UNCONTROLLABLE STRESS ON IMMUNE FUNCTION IN HUMANS

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Abstract

Title of Dissertation: Differential Effects of Controllable and Uncontrollable Acute Stress on Immune Function in Humans

Carol Silvia Weisse, Doctor of Philosophy, 1989

Dissertation directed by: Andrew S. Baum, Ph.D., Department of Medical Psychology

The mechanisms by which stress impacts on health are not clearly understood. Research suggests that susceptibility to disease may increase during stress because of stress-induced deficits in the immune system; however, empirical evidence is lacking for a causal relationship between stress and altered immune function in humans. This study examined the effects of an acute, thirty minute laboratory stressor on aspects of immune function in 24 men. Also examined in this study was whether behavioral control over the stressor would mediate stress-effects. The stressor consisted of mild (2.5 mA) electric shock and loud (100 dB) pure tones (3000 Hz) administered in an unpredictable, intermittent fashion. During stress sessions, only half of the subjects were able to control the stressor. Subjects with control were yoked to subjects who could not control the stressor so that both groups received stressors of identical intensity and duration. Stress responses were assessed using behavioral and self-report measures obtained at critical points throughout stress and non-stress conditions. Aspects of immunologic function were assessed across conditions by measuring changes in lymphocyte proliferation to concanavalin A (Con A)

and phytohemagglutinin (PHA) and by measuring changes in percentages of immune cell populations including lymphocytes, granulocytes, monocytes, and lymphocyte subpopulations. Results revealed that exposure to the uncontrollable stressor caused changes in mood and interfered with anagram performance, but it did not affect immune function. On the other hand, exposure to controllable stress did not alter mood or task performance but did result in lowered lymphocyte proliferation to Con A. Post-stress percentages of monocytes were also lower in subjects exposed to controllable stress than in subjects exposed to uncontrollable stress. Results suggest that acute stress can alter aspects of immune function in humans, and further, they underscore the importance of stressor controllability in mediating stress effects on the immune system. Results are discussed in terms of behavioral response demands. Differences between acute and chronic stress are highlighted, and future research topics are outlined.

DIFFERENTIAL EFFECTS OF CONTROLLABLE AND UNCONTROLLABLE ACUTE STRESS ON IMMUNE FUNCTION IN HUMANS

by

Carol Silvia Weisse

Dissertation submitted to the Faculty of the
Department of Medical Psychology Graduate Program of
the Uniformed Services University of the Health Sciences
in partial fulfillment of the requirements for the degree of
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Dedication

To Steven... for supporting this project in so many ways: for accompanying me to the lab on Saturday and Sunday mornings... for sitting up with me late at night as I worked on the computer... for helping me label tubes... and enter data... and make graphs... for always believing in me... and for never questioning the importance of this project.

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I am grateful to Carlos Pato and Robert Littman for their dedication to this project. Without them, this project would not have been possible nor would it have been completed in such a timely fashion. Special thanks are extended to Cathy McAllister for welcoming me into her laboratory, for teaching me the necessary immunologic techniques, for proofing this manuscript, and for always being there with words of encouragement. I would like to acknowledge Jean Collison for her computer systems expertise, and I would like to extend my gratitude to Tom Shimotake, Marge Ornestein, and Laurel Beer, not only for their technical assistance, but for their moral support throughout the project. They provided a critical liaison between myself and the NIMH.

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Introduction

Considerable evidence suggests that stress can affect immune processes. Within the last decade, several review papers and chapters examining the relationship between stressful events and various immune states have been published, and nearly all have concluded that there is strong support for such a relationship in animals and in humans (Borysenko & Borysenko, 1982; Calabrese, Kling, & Gold, 1987; Jemmott & Locke, 1984; Kelley, 1980; Schindler, 1985; Stein, 1985; Tecoma & Huey, 1985). Animal studies consistently find a variety of immune changes following an array of stressors including electric shock, restraint, noise, rotation, separation, crowding, isolation, and cold water immersion (e.g., Harmsen & Turney, 1985; Jensen, 1969; Kandil & Borysenko, 1987; Laudenslager, Reite, & Harbeck, 1982; Monjan & Collector, 1977; Okimura, Ogawa, & Yamauchi, 1986). Most of these animal studies report reduced immune function, particularly lowered lymphocyte proliferation in response to mitogens, following exposure to stressful stimuli. Human studies have also related altered immunity to stressful events. During events such as bereavement, family illness, divorce, unemployment, and academic examination periods, humans tend to exhibit signs of altered immunity (e.g., Arnetz et al., 1987; Bartrop, Lazarus, Luckhurst, Kiloh, & Penny, 1977; Linn, Linn, & Jensen, 1984; Workman & La Via, 1987). Human studies, like animal studies, often report lowered lymphoproliferative abilities during stressful events.

While many stressful stimuli have been related to altered immune states in animals and in humans, only animal studies provide clear evidence for a causal relationship between stress and associated immune

changes. In most animal studies of stress and immunity, investigators randomly assign animal subjects to experimental groups and collect critical baseline data. This approach allows examination of immune states before and after a stressor is administered and enables conclusions to be drawn about stress effects on the immune system. Stressful events may cause immune changes in people, but this has not been adequately investigated in humans. Human studies differ from animal studies, particularly with respect to methodological design. For example, many human studies are correlational, and comparisons are often drawn between individuals undergoing specific life events (e.g., bereavement, unemployment) and individuals who are not experiencing the particular events in question. However, human studies rarely examine the effects of stress manipulations on immune function. Whether stress actually causes immune changes to occur or whether stress-related immune differences are the result of other factors, such as health-impairing behaviors that often accompany stressful life events, cannot be determined from correlational studies.

Although animal experiments provide strong support for a causal relationship between stress and altered immunity, these studies are not easily compared to human studies. It is difficult to measure how stressful an event is to an animal, and these studies typically use stressors that are intense and readministered over several hours, days, weeks, even months. Animal studies provide support for a causal relationship between stressful events and immune changes in several species, but they may not adequately address whether a similar relationship exists in humans. Therefore, one purpose of this project was to investigate whether an acute laboratory stressor could cause a change

in immune function in humans. This project also examined whether having behavioral control over a stressor could mediate stress-induced immune effects.

Clearly, both animal and human studies offer important information about the relationship between stress and immunocompetence. Animal studies provide certain advantages over human studies but also possess certain limitations; therefore, both animal and human studies will be reviewed, but separately, in this chapter. Because studies of stress and immunity have not used consistent measures of immunocompetence, a brief discussion of how immunocompetence is measured is provided first. Next, animal studies are reviewed, followed by a review of human research. Finally, hypotheses about human stress and immune function are generated, and the study is outlined.

Measures of immunocompetence

While this section is not intended to describe all potential measures available for assessing immunocompetence, its purpose is to provide the reader with a brief overview of the measures that have been commonly used in both human and animal stress studies. For discussion of the clinical relevance of these tests of immunocompetence, see Stites (1984a, 1984b).

Cells of the immune system, collectively termed leukocytes or white blood cells, are broadly divided into three main classes: lymphocytes, monocytes, and granulocytes. Functional differences exist among these diverse classes of cells, and further distinctions are often drawn among leukocyte subpopulations on the basis of these differences. For example, lymphocytes may be subdivided into B cells, T-helper cells, T-suppressor cells, T-cytotoxic cells, and natural killer cells,

while granulocytes include neutrophils, basophils, and eosinophils. Because of the many types of immune cells and the fact that these various leukocytes perform many different processes, immune function may be measured in a number of ways. Quantitative as well as qualitative measures are used to assess immunocompetence in animals and in humans. These techniques include methods that count and challenge nearly all leukocyte populations.

A number of functional assays are used to measure actual cell responses in studies of stress and immunity. Leukocytes possess a variety of capabilities including the ability to proliferate in response to antigens, to move toward the site of infection (chemotaxis), to engulf foreign pathogens (phagocytosis), to aggregate (accumulation) and adhere to infected surface areas, to destroy through specific lytic activities as in tumoricidal activity (cytotoxicity), to bind to surface membranes (adherence), and to generate humoral regulatory or growth factors (e.g., antigen specific antibodies, interferon, and interleukins). Not all cells are capable of all of these activities, so differences in cell functions may require different assays. Assays are available to measure all of these aspects of immunologic function.

While most immune assays are performed <u>in vitro</u> and may measure the activities of cells independently from one another, there are tests of immunocompetence which are measured <u>in vivo</u> and assess the coordinated actions of a variety of cell types and cell products. For these reasons, <u>in vivo</u> measures may be more meaningful measures of immunocompetence because they consider the system as a whole. Skin tests for delayed hypersensitivity and measures of antibody production following exposure to antigen, as in a vaccine, are examples of <u>in vivo</u> measures

of immune function. An additional *in vivo* index of immune function involves monitoring graft-versus-host (GVH) responses in order to determine how well an organism can reject non-histocompatible tissue grafts. This rejection process is immunologically mediated and is often reduced in immunocompromised animals.

Most studies of stress and immunity rely on proliferation or cytotoxicity assays. Lymphocytes are stimulated to divide *in vitro* using plant lectins ("mitogens") such as phytohemagglutinin (PHA), concanavalin A (Con A), or pokeweed mitogen (PWM) or using allogeneic lymphocytes from non-histocompatible donors, as in a mixed lymphocyte reaction (MLR) test. Mitogens and "non-self" lymphocytes act as antigenic substances and activate lymphocyte proliferation *in vitro*. Other immune cells such as natural killer cells and macrophages (mature monocytes) can be challenged with tumor cells *in vitro* to assess killing activity as opposed to proliferative abilities. Both lymphocyte proliferation and natural killer cytotoxicity assays are used with great frequency in human and animal studies of stress and immune function.

Some studies of stress and immunity measure immune status as opposed to immune function; in other words, they measure quantities of particular immune cells or cell products instead of the activities of these cells. A crude quantitative measure of immune status is the total white blood count (WBC). The WBC represents the number of total leukocytes per cubic millimeter of blood. Normal values are extremely variable (e.g., 5,000-10,000 cells/mm^3), and deviations from this norm are difficult to interpret, particularly without a differential analysis. The differential analysis is a breakdown of the WBC into percentages of lymphocytes, monocytes, and granulocytes, therefore

enabling calculations to be made of these specific cell populations. Additional techniques (e.g., flow cytometry) are available for more detailed calculations of specific leukocyte subpopulations.

Immune status is also measured by assessing antibody status of an organism. Antibodies are serum proteins, also called immunoglobulins, that are secreted by B lymphocytes in five different classes: IgA, IgD, IgE, IgG, and IgM. Antibody-secreting capabilities may be altered in certain disease states and, therefore, can be considered a humoral measure of immunocompetence. For example, elevations in autoantibodies often indicate autoimmune processes. On the other hand, elevations of specific antibodies to latent viruses such as Herpes simplex virus (HSV) or Epstein-Barr virus may indicate reinfection or reactivation of the virus and, hence, suggest lowered immunocompetence (Glaser & Gottlieb-Stematsky, 1982).

In the present study, the term "immune status" will be reserved to denote immunocompetence as measured by quantitative techniques such as the white blood count (WBC), the differential analysis, and cell counts determined through flow cytometric techniques while "immune function" will refer to immunocompetence as determined by functional assays such as natural killer cell activity, chemotaxis, phagocytosis, and lymphocyte proliferation. These assays that measure immune status and immune function are listed in Table 1.

Insert Table 1 about here

Animal studies of stress and immunity

Of a wide variety of laboratory stressors, electric shock has most consistently been used to evoke changes in immune function. Wistar and Hildemann (1960) exposed mice to a two week regimen of daily (6 hour/day) signalled, escapable shock and found that graft-versus-host responses were lower in these mice than in control mice that were not exposed to shock. In another study, several months of exposure to signalled, escapable electric shock was shown to reduce lymphocyte responses to mitogens. Odio, Golisek, Brodish, and Ricardo (1986) exposed rats to 2-4 hour, daily exposures of signalled, escapable shock. After six months, rats were bled and lymphocyte responses to Con A and PHA were assessed. Lymphocyte responses to these mitogens were lower in rats after six months of avoidance shock than in control animals. This study and the study by Wistar and Hildemann (1960) did not collect immune data throughout the course of the study making it impossible to determine how long a stressor period was necessary to elicit immune changes. However, both studies showed that immune function was reduced by prolonged exposure to signalled, escapable electric shock.

Keller, Weiss, Schleifer, Miller, and Stein (1981) also found lowered immune function in rats exposed to unsignalled, electric shock, yet the change was noted following 20 hours of shock and the shocks were inescapable. Shocks were administered to rats at a frequency of one per minute over a period of twenty hours. Following this stress period, lymphocyte responses to PHA were significantly lower in experimental animals than in control rats. A marked lymphocytopenia was also noted in experimental animals, and a higher intensity shock produced

greater depressions in lymphocyte responsivity, suggesting that reductions in lymphocyte responses were caused by the electric shock and were a function of the intensity of the stressor.

Changes in immunocompetence have been observed in animals following even shorter exposures to electric shock. Harmsen and Turney (1985) administered 3 hours of intermittent, inescapable shocks (approximately one shock per minute) to rats and tested three aspects of neutrophil function: adherence, accumulation, and phagocytosis. Neutrophils from stressed rats exhibited poorer accumulation at a zymosan (yeast cell fragment) injection site compared to those from control rats. However, phagocytic activity and adherent capabilities of the neutrophils were actually higher in stressed rats.

Numbers of circulating lymphocytes have also been altered in animals by brief periods of stress. Marsh, Lavender, Chang, and Rasmussen (1963) trained monkeys to press a key at a steady rate to avoid a shock to the tail which would be delivered every 10 seconds if the key was not pressed. Monkeys exhibited lowered lymphocyte numbers within 3 hours of shock avoidance stress. Although lymphocyte responses were not measured in this study, lymphocyte responses have been shown to be altered by brief stress as well. Shavit, Lewis, Terman, Gale, and Liebeskind (1983) reported lowered lymphocyte responses to PHA and Con A in rats after as little as 20 minutes of intermittent, inescapable electric shock. Studies by Harmsen and Turney (1985), Marsh et al. (1963), and Shavit et al. (1983) suggest that stress can bring about fairly rapid changes in the immune system and that intensive, long exposure periods of stress are not necessary to induce significant changes in immune functioning.

Studies examining the effects of electric shock on immunocompetence are difficult to compare because the predictability and controllability of the shocks varied greatly across studies. While some studies used signalled shock-avoidance paradigms and provided animals with both a controllable and predictable stressor (Marsh et al., 1963; Odio et al., 1986; Wistar & Hildemann, 1960), other studies used unpredictable and uncontrollable shock (Harmsen & Turney, 1985; Shavit et al., 1983). One study has shown stressor controllability to be an important mediator of stress-induced immune changes (Laudenslager, Ryan, Drugan, Hyson, & Maier, 1983). In this study, investigators administered 80 shocks (approximately one per minute) to three groups of rats; one group could terminate the shocks, one group was yoked to the first group and received identical shocks except the shocks were inescapable, and the third group was a control group that was placed in the experimental apparatus but was not administered shock. A group of home cage controls was also included. The study revealed that lymphocyte responses to PHA were significantly reduced in the rats unable to escape exposure to shocks but not in the rats receiving the same intensity and duration of escapable electric shock. Group differences in responses to Con A were not as great, but occurred in the same direction as responses to PHA.

Laudenslager et al. (1983) reported lower lymphocyte responsivity in rats receiving uncontrollable shocks, but not controllable shocks. However, studies by Odio et al. (1986) and Wistar and Hildemann (1960) both reported that lymphocyte responsivity was lower in rats following exposure to controllable shocks. One possible explanation for the differences found between these studies may be that con-

trollability over a stressor is protective against acute stressors but not against chronically administered stressors. Laudenslager et al., (1983) administered shocks for approximately 80 minutes. Odio et al. (1986) and Wistar and Hildemann (1960), on the other hand, exposed rats to long-term stress, lasting 2 weeks and 6 months respectively. Thus, control may be an important modulating variable of stress-induced changes of lymphocyte responsivity to mitogens only when exposure to the stressor is not prolonged.

Stressors other than electric shock have been shown to elicit changes in immunocompetence among animals. For example, Pavlidis and Chirigos (1980) found immune changes to occur following 18 hours of restraint stress. In this study, macrophage tumoricidal activity was measured in restrained mice and in controls for a total of 18 hours. By collecting immune measures from two mice every two hours, Pavlidis and Chirigos found that macrophage tumoricidal activity was lower in experimental mice than in controls, but not until after 18 hours of restraint. Kandil and Borysenko (1987) also examined immune responses throughout a lengthy stressor period and found natural killer activity to be lower two weeks after a six day period of rotational stress. In this study, rats were placed, while in their cages, on a turntable programmed to rotate at ten minute, intermittently dispersed intervals each hour, 24 hours each day, for a total of six days. Natural killer cell activity was measured in rats on days 4,7,10, 13, 20, and 27 after rotational stress. Natural killer cell activity was lower only on days 13, 20, and 27 following rotational stress.

Natural killer activity has also been shown to decline in mice after an 8-day regimen of immersion in cold water (Aarstad, Gaudernack,

& Seljelid, 1983). Aarstad et al. (1983) exposed mice to the cold water for 5 minute periods, two times a day and repeated this schedule over the course of 8 days. Natural killer cell activity was measured in mice each day. Declines in natural killer activity were noted after 5 days of the cold water stress. All of these studies suggest that long periods of stress may be necessary to evoke changes in macrophage and in natural killer tumoricidal activity.

Comparisons across all animal studies are difficult because different species, measures of immunocompetence, stressors, and exposure schedules were used. Most stressors used in these studies were intense and were administered over lengthy periods of time. Furthermore, because many of these studies examined only one measure of immunocompetence and collected the measure at the end of a long stressor period, it is impossible to determine if immune responses other than those measured were being affected by the various stressors and further, when changes occurred. Studies that performed sampling across time suggest that changes in natural killer and macrophage activity occur gradually after fairly lengthy stressor periods (Aarstad et al., 1983; Kandil & Borysenko, 1987; Pavlidis & Chirigos, 1980), but that neutrophil function, changes in lymphocyte numbers, and lymphocyte responses to mitogens may be altered much more rapidly following exposure to stressful stimuli (Harmsen & Turney, 1985; Marsh et al., 1963; Shavit et al., 1983).

Noise, isolation, and crowding are additional stressors known to evoke immune changes in animals. Crowding and isolation have been related to changed immunocompetence (Jessop, Gale, & Bayer, 1987; Joasoo & McKenzie, 1976); however, these stressors are associated with

increases or decreases in exposure to pathogens from other animals. Therefore, altered immunity under these conditions is difficult to interpret. Noise, on the other hand, represents a stressor that is not associated with differences in exposure to pathogens. Susceptibility to viral infections is altered in mice following exposure to three hours of constant, high intensity (123 dB) sound (Jensen & Rasmussen, 1963), Deficits within the immune system have been proposed as mechanisms for altered susceptibility to viral pathogens in mice undergoing audiogenic stress (Chang & Rasmussen, 1965; Jensen, 1969). Chang and Rasmussen (1965) exposed mice to daily, 3 hour sessions of 120 dB noise. They noted that interferon production was depressed throughout the 6-8 day stressor period even though animals had been exposed to a virus to stimulate interferon production. In addition to changes in interferon production, researchers have also found that leukocyte numbers change following exposure to noise stress. For example, Geber (1966) reported that numbers of eosinophils declined in mice following 30 minutes of constant noise (approximately 83 dB). In addition, Jensen (1969) administered 24 hours of 120 dB sound stress to mice and found a marked leukopenia within two hours. These studies all reported fairly rapid changes in leukocyte numbers, but none examined functional changes in the immune system following noise stress.

One study has reported altered immune function in mice following exposure to noise stress. Monjan and Collector (1977) exposed mice to 1-3 hour daily sessions of 115 dB noise and found that during the first 20 days of this regimen, lymphocyte responses to LPS and Con A were lower in experimental mice than in controls, but that during the next 20 days of noise, lymphocyte responses of these mice actually rose

above responses of controls. This study suggests that acute stressors may produce different patterns of immune change than chronic stressors. Noise stressors do appear to elicit fairly rapid changes in immune function, but initial changes may not reflect more long-term effects.

In summary, animal studies not only provide strong support for a causal relationship between stress and altered parameters of immunity, but these studies suggest that regardless of what kind of stressor is administered and which measure of immunologic function is examined, stressors elicit changes in a spectrum of immune responses. Few studies fail to find some immune change in response to stressful stimuli. Animal studies also suggest that immune changes that occur following exposure to acute stressors may be different and even opposite to those changes noted after exposure to more lengthy stressor schedules (Monjan & Collector, 1977). Evidence suggests that some immune changes (e.g., lymphocyte responses to mitogens, altered leukocyte numbers, neutrophil function) may change more rapidly than others (e.g., macrophage tumoricidal activity, natural killer activity). Stressor controllability also appears to modulate acute stress-induced immune changes in animals (Laudenslager et al., 1983), but this has not been extensively studied. Most stressors used in the animal studies of stress and immunity reviewed here were uncontrollable (e.g., restraint, noise, rotation, and cold water immersion).

While animal studies suggest that acute stressors are capable of causing changes in immunity and that control may be an important mediator of this relationship, these issues have not been investigated in humans. Whether acute stress alters human immunocompetence is not

clear, and although control has been shown to mediate stress-responding in humans (e.g., Cohen, Evans, Stokols, & Krantz, 1986; Glass & Singer, 1972), the role control plays in the relationship between stress and immunocompetence has not been examined closely. The next section provides a review of human studies which have examined the relationship between stress and immunity.

Human studies of stress and immunity.

Studies of individuals during marital disruption, bereavement, unemployment, and academic examination periods suggest that aspects of immunologic function are altered in people undergoing stressful life events. Kiecolt-Glaser et al. (1987) studied 38 women following divorce or marital separation and compared a variety of immune measures from these women to measures from 38 married controls. Divorced/separated subjects showed greater signs of distress, chiefly depression, as evidenced by higher scores on the Brief Symptom Inventory. In addition, women experiencing marital disruption exhibited lower lymphocyte responses to PHA, lower percentages of T4 helper cells, lower percentages of natural killer cells, and higher titers of antibodies against Epstein-Barr virus (EBV). No differences were found in the percentages of T8 suppressor cells, ratios of T4 helper to T8 suppressor cells, or for lymphocyte responses to Con A. While divorced/separated women exhibited some signs of lowered immunocompetence, they also reported greater alcohol consumption and had higher levels of transferrin in their blood, an indicator of poor nutrition. Therefore, it is impossible to know whether stress, or behavioral factors such as diet or alcohol consumption, or even other factors related to the separation were responsible for the occurrence of these immune

differences.

Arnetz et al. (1987) studied 17 unemployed women and 8 employed controls to determine if unemployment was related to decreased immune function. Lymphocyte responses to PHA and PPD were measured in all subjects; however, sampling procedures were not symmetrical and subjects were studied at different times of the year. The women had been unemployed for nine months and lymphocyte responses to PHA and to PPD were measured only once at this time. Although lymphocyte responses were found to be lower in unemployed women than in employed controls, the data are difficult to interpret. No baseline measures were available on these women, and while smoking and alcohol were monitored, diet and exercise were not. Furthermore, investigators did not measure whether unemployed women were indeed experiencing greater stress than employed controls. Lymphocyte responses were lower in unemployed women, yet investigators did not relate these immune differences to symptoms of stress.

Studies of bereavement provide similar support for a relationship between stressful life events and altered immunity, but like the studies of marital separation and unemployment, the data are correlational and do not provide evidence for a causal relationship. Four studies have examined immunocompetence in individuals during bereavement (Bartrop, Lazarus, Luckhurst, Kiloh, & Penny, 1977; Irwin, Daniels, Bloom, & Weiner, 1986; Irwin, Daniels, Smith, Bloom, & Weiner, 1987; Schleifer, Keller, Camerino, Thornton, & Stein, 1983), and while these studies suggest that decreased immune activity can occur during bereavement, they also suggest lowered immune function may be related to how an individual reacts to the death of a loved one.

Bartrop et al. (1977) followed 26 people immediately after the accidental or illness-related death of their spouse and compared immune data from these individuals to 26 control subjects, matched for age, sex, and race. Bereaved subjects did not differ from controls 1-3 weeks following the death of their spouse, but they did have significantly lower lymphocyte responses to PHA and Con A six weeks later. Behavioral and psychological data were not reported, but a wide variety of immune measures were. While bereaved subjects did not differ from controls in T and B cell numbers, antibody titers, autoantibodies, or in delayed type hypersensitivity at either time period, they did exhibit lower lymphocyte responses to PHA and Con A six weeks after their spouses had died.

Schleifer et al. (1983) reported similar findings in another study of bereavement. In this study, 15 spouses of women who were terminally ill with metastatic breast cancer were followed, and lymphocyte responses to PHA, Con A, and PWM were tested at three time periods: before death of the spouse, within one month after the death, and during a four to fourteen month follow-up. Baseline data were available for these subjects but matched controls were not studied. Results of this study showed that responses to all three mitogens were lower post-bereavement than pre-bereavement, and a trend toward baseline was observed during the follow-up period. Neither numbers nor percentages of T and B cells changed throughout the study, thus confirming the results of Bartrop et al. (1977). However, differences were noted by Schleifer et al. (1983) much earlier into the bereavement period than were noted by Bartrop et al. (1977).

One possible explanation for the time differences between these

two studies may be that the subjects studied by Schleifer et al. (1983) were experiencing greater stress because they had been dealing with bereavement issues longer than the subjects in the study by Bartrop et al. (1977). Some of the subjects studied by Bartrop et al. (1977) lost their spouses suddenly in accidents, while subjects studied by Schleifer et al. (1983) were all aware of their spouses' impending death. One can only speculate about possible differences in actual time spent grieving by these subjects because stress measures were not included in either study. In addition, Schleifer et al. (1983) did not study matched control subjects. Therefore, while the studies by Bartrop et al. (1977) and Schleifer et al. (1983) suggest lower lymphocyte responses during bereavement, the role of stress in directing the relationship between bereavement and lower immune responses was not directly assessed.

Without measuring symptoms of stress during events such as unemployment or bereavement, it is difficult to make assumptions about a stress-imune link. Some studies provide evidence that individual responses to stressful events may mediate whether differences are noted in immune function (Irwin et al., 1986, 1987; Linn, Linn, & Jensen, 1984; Workman & La Via, 1987). Irwin et al. (1986) compared natural killer activity in women who had been widowed for less than six months, women whose husbands were diagnosed with terminal lung cancer, and women whose husbands were healthy. The study revealed that natural killer activity of the bereaved women was not significantly different from the natural killer activity of the other groups. However, natural killer activity was related to symptoms of depression. The data from this study and from follow-up research (Irwin et al., 1987) suggest

that immune disturbances may depend on how the event (e.g., spouse's death) is interpreted by the individual.

Other studies of humans during stressful events have confirmed the importance of individual response patterns in determining stress-related immune differences. Linn, Linn, and Jensen (1984) examined 49 men who had recently experienced a death or serious illness in the family and compared a variety of immune measures from these men (e.g., serum antibody levels of IgG and IgA, delayed type hypersensitivity (DTH), mixed lymphocyte responses (MLR), neutrophil migration, lymphocyte responses to PHA) to measures from 49 control subjects who had not recently experienced a similar event. Men who had recently experienced a stressful life event did not differ immunologically from control subjects except in levels of IgA; all other immune measures were comparable between groups. However, when subjects were split into groups of high and low depression by scores from the Hopkins Symptom Checklist, the more depressed subjects exhibited lower lymphocyte responses to PHA than the less depressed group of men. Perceived stress was also higher in the more depressed group. Thus, lymphocyte responses to PHA were lower in subjects reporting greater stress and depression regardless of whether they had recently experienced a stressful event. This study provides evidence suggesting that comparisons should not be drawn between individuals exposed to a stressful event with individuals who have not experienced a similarly defined event unless measures are included assessing how individuals respond to the stressor involved.

A study of examination stress by Workman and La Via (1987) lends credence to the notion that individual responses to events may mediate

the relationship between stress and immunity. While a number of studies investigating immune function in students taking exams show higher stress and lower immune function during exam periods (Dorian et al., 1982; Glaser, Rice, Speicher, Stout, Kiecolt-Glaser, 1986; Kiecolt-Glaser et al., 1984), Workman and La Via (1987) related differences in immune function to the manner with which students responded to exam stress. They examined 15 students taking the national medical board exam and 15 age/sex matched control subjects for differences in lymphocyte responses to PHA. In addition, all subjects in the study completed the Impact of Events Scale (IES) to measure recent stress levels and style of responding to these recent stressors. Two subscales of the IES provided measures of avoidance (e.g., the extent to which an individual made cognitive attempts to avoid a recent stressor) and intrusion (e.g., the extent to which the stressor intruded into the individual's experience). The results of the study indicated that subjects taking the exam were more highly stressed and had significantly lower lymphocyte responses to PHA compared to controls. Furthermore, exam students with higher intrusion than avoidance scores exhibited lower lymphocyte responses than students who did not have higher intrusion than avoidance scores, suggesting that cognitive coping responses may have mediated stress-related immune differences.

In summary, studies of divorce, bereavement, unemployment, and examination periods all suggest a relationship between stressful events and immune responsivity, but none of these studies allow causal inferences to be drawn. These studies all used correlational designs and examined events typically associated with long-term consequences (financial, social, behavioral). One question that is raised by these stud-

ies is whether or not stress actually caused the lowered immune states or if other factors were responsible for the changes that were noted. Most studies failed to include direct measures of stress making it impossible to validate stress responses and examine individual differences. In addition, behavioral events were not routinely reported.

Many changes known to occur during stressful events may have been responsible for immune differences. Sleep patterns (Palmblad, Petrini, Wasserman, & Akerstedt, 1979), nutritional status (Beisel, Edelman, Nauss, & Suskind, 1981), exercise (Simon, 1984), alcohol use (VanThiel, 1983) and cigarette smoking (Holt & Keast, 1977) are all known to have effects on the immune system, particularly on the ability of lymphocytes to respond to mitogens, and these health-impairing behaviors are also likely to increase during stressful events (e.g., Clayton, 1979). Therefore, while stressful events have been related to altered leukocyte numbers, decreased lymphocyte responses, and lower natural killer activity in humans, it cannot be concluded on the basis of these studies whether or not stress actually caused the differences that were observed. Furthermore, these studies provide little information regarding the time frame with which changes in immune function occur.

Studies of acute stress and its direct effects on immunologic function may provide stronger evidence for a stress-immune relationship in humans, particularly when immune measures are collected before health-impairing behaviors are initiated. Studies of acute stress and immune function may also offer support for a causal relationship between stress and immunocompetence because they could be more easily designed to yield experimental data. In addition, these studies would

provide information about the time course involved in eliciting immune change. Unfortunately, few studies have examined the effects of acute stressors on immunologic function in humans.

Exercise studies provide some evidence for acute stress-induced changes in human immunologic states (Brahmi, Thomas, Park, & Dowdeswell, 1985; Eskola et al., 1978; Hedfors, Holm, & Ohnell, 1976; Steel, Evans, & Smith, 1974). Brahmi et al. (1985) measured numbers of lymphocytes, numbers of natural killer cells, and natural killer cell activity in 15 subjects before and after a cycling exercise that lasted approximately 20 minutes. All subjects exercised to their maximum VO_2. These subjects were not studied under similar non-exercise conditions, nor were subjects who did not exercise included as a control for changes which may have occurred as a function of time or laboratory procedures. Results revealed that total T and T4 lymphocyte numbers were decreased immediately following exercise, while natural killer cell numbers and activity actually increased. Two hours after exercise, natural killer activity dropped significantly. Twenty hours following the exercise routine, all immune measures returned to baseline levels. The results are difficult to interpret, however, without data from control subjects. Changes may have occurred due to circadian rhythms or merely due to blood draw procedures.

Steel et al. (1974) studied six subjects along with 4 controls to determine whether immune changes would occur after a brief running exercise. All subjects exercised for 10 minutes, and maximum VO_2 was not measured. Results revealed that increases in lymphocyte numbers occurred in response to exercise, and that these lymphocytes were mostly B cells. Exercise subjects exhibited a significant increase in

lymphocyte numbers and were returned to baseline levels within 45 minutes of the run; measures collected at 2 and 4 hours post exercise were not different from baseline.

Hedfors et al. (1976) also reported an immediate lymphocytosis following a brief exercise period. In this study, subjects were catheterized and allowed to sit for 30 minutes before beginning a cycling exercise. This allowed subjects to adjust to the blood sampling procedure and allowed investigators to collect a reliable resting blood sample. Subjects performed bicycle ergometer work until a heart rate of 150 beats per minute was achieved. Total exercise time was 15 minutes. The study revealed both quantitative and qualitative changes in immunocompetence following exercise, but control subjects were not studied. Subjects exhibited increases in T and B lymphocytes, and the responses of lymphocytes to PHA, Con A, PWM, and PPD were lower after exercise compared to pre-exercise levels. This study suggests a change in immune function as well as immune status can occur after a very brief exercise period, but because control subjects were not studied, one can similarly conclude that the changes observed in immune function were the result of the catheterization procedure or even the result of circadian rhythms.

While Hedfors et al. (1976) report lowered lymphocyte responsivity to mitogens following exercise, Eskola et al. (1978) report lymphocyte responsivity is not altered after exercise. Eskola et al. (1978) measured immune function in 4 subjects who completed a 35 minute, 7 kilometer run and found no changes in lymphocyte responsivity. This study failed to include control subjects as well, and the subjects in this study were trained athletes and may not have been under as great

stress as the untrained subjects studies by Hedfors et al. (1976). Because of these differences across exercise studies, comparisons are difficult to make.

Although exercise studies do provide some evidence for stress-induced quantitative and functional changes in immunocompetence, conclusions about stress are difficult to draw. The stressfulness of the exercise regimens was never measured in these studies. In addition, subject sample sizes were small, and only Steel et al. (1974) included control subjects. Some studies included trained athletes as subjects while others did not, and the findings from the studies are not all consistent (e.g., increases vs. decreases in lymphocyte numbers). It is also difficult to compare how a physical stressor such as exercise might compare to a more cognitive stressor such as an examination.

Exercise may very likely represent a stressor that is very different than a stressor such as an oral or written exam, which is often associated with similar hormonal patterns but without the associated increase in activity level. Landmann et al. (1984) report immune changes in humans following a brief cognitive task. Subjects were also put through a physical exercise regimen immediately after the cognitive task was completed; therefore, some of the effects of the psychological stressor may have been masked. In this study baseline blood samples were collected from 15 healthy subjects and then subjects were administered the Stroop cognitive task. Immediately after subjects completed this task, a second blood sample was collected. Next, subjects were exercised by bicycle ergometry for 15 minutes, and an additional blood sample was obtained. Blood samples were assayed for leukocyte popula-

tions, catecholamine levels, and cortisol. Heart rate and blood pressure data were also collected.

The investigators found that numbers of monocytes, natural killer cells, and B cells increased significantly after the 8 minute cognitive task, and all other leukocyte populations (e.g., helper and suppressor T-cells, granulocytes) increased following the physical stressor. Blood pressure and heart rate were elevated in response to the Stroop, but catecholamine levels and cortisol did not increase until after physical exercise. It is impossible to know, however, whether the changes observed following exercise were from the exercise alone, or whether they represented a cumulative effect of the Stroop and exercise together. Without control subjects, it is impossible to tell whether changes were an artifact of time or the procedures. Therefore, the data from this study only provide preliminary evidence that a "psychological" stressor can elicit rapid immune changes in humans. This study did not allow subjects to return to baseline before beginning the exercise regimen, and thus, the complete time frame of immune changes in response to the cognitive stressor alone was not determined. In addition, only quantitative measures were included, and a relationship between changes in leukocyte subpopulations and changes in immune function has not been well established.

The study by Landmann et al. (1984) provides the strongest evidence for a causal relationship between psychological stress and altered immunocompetence because the investigators experimentally administered a cognitive laboratory stressor; health-impairing behavioral factors were not relevant. Subjects were randomly assigned to groups and baseline data were available before the stress period. However,

control subjects were not included and the conclusions one can draw from the data are limited. Clearly, further definitive research including adequate control groups is needed.

Hypotheses

Research examining stress and immunity in animals indicates that stressful events, particularly those that are chronic and unavoidable, can cause altered immunocompetence. However, evidence for a causal relationship between stress and altered immune states in humans is less clear. There is a paucity of human experimentation investigating the effects of psychological stressors on immunocompetence. Information about stress and its relationship to the human immune system comes mainly from correlational studies of individuals undergoing long-term stressors. From these studies, one can only speculate about the causes of altered immunocompetence. A number of questions remain. For example, can a psychological stressor cause humans to exhibit altered immune status or function? Furthermore, can altered immune states result from an acute stressor, or are chronic events necessary for such changes to occur?

While animal studies lend credence to the existence of a causal relationship between stress and immunity, they do not allow examination of important cognitive factors which may play a mediating role in the relationship between stress and human immunocompetence. There is some evidence, however, that stressor controllability mitigates acute stress- effects on immunocompetence in animals. Considerable research supports the importance of control in mediating stress responses in humans, and questions have been raised concerning the importance of control in mediating the relationship between stress and immunity in humans (Jemmott & Locke, 1984). However, this area remains unexplored, and it is not known whether psychological factors such as control can modulate the relationship between stress and immune function in humans.

This experiment examined the questions just raised. The effects of both controllable and uncontrollable stress on immune function were investigated in humans, and three hypotheses were tested. First, because unpredictable aversive stimuli are known to elicit stress responses, it was hypothesized that subjects exposed to intermittent noise and electric shock would exhibit signs of stress and altered immune function (e.g., lower lymphocyte responses to Con A, PHA). Second, it was hypothesized that subjects unable to exert behavioral control the noise and electric shock would exhibit greater signs of stress and lower immune function than subjects allowed to control the noise and electric shock. Finally, it was hypothesized that symptoms of stress would be inversely related to the magnitude of immune function. In other words, it was predicted that following exposure to the noise and electric shock, subjects exhibiting greater signs of stress would also exhibit lower immune function than subjects exhibiting fewer signs of stress.

Method

Overview and design

A combination of noise and electric shock served as the stressor in this study. While this study examined two groups of individuals, a within subjects design allowed subjects to serve as their own controls. All subjects participated in a baseline (no stress) session to control for the effects of time and blood drawing procedures. In addition, subjects participated in one of two experimental sessions where they were randomly assigned to receive controllable or uncontrollable stress. Subjects assigned to the controllable stress group were yoked to subjects from the uncontrollable stress group so that all paired subjects received identical amounts of the noise/shock stressor. Two subjects, one from each group, were run at a time. Subjects were scheduled so that one subject was run through their baseline session while another was run through their experimental session. Therefore, baseline sessions occurred at the same time of day as experimental sessions and lasted for the same duration of time. In addition, baseline sessions were counterbalanced with experimental stress sessions so that half of the subjects were run through their experimental session before their baseline session. Figures 1 and 2 illustrate the time line of events for baseline and experimental sessions respectively. Sessions were as similar as possible and were divided into four events: 60 minute initial rest period, exposure to 30 minute noise/shock stressor or 30 minute rest period, 20 minute performance task, and final 90 minute rest period (see Figures 1 and 2).

Insert Figures 1 and 2 about here

Apparatus

The apparatus used in this study was originally developed by Brier et al. (1987) for use in a human study examining the effects of uncontrollable and controllable noise on mood, psychophysiology, and neuroendocrine patterns. The apparatus was an adaptation from earlier studies by Hiroto (1974) and Hiroto and Seligman (1975) of learned helplessness. A PDP-11 computer administered the stressor to subjects in both groups. The stressor consisted of approximately 30 minutes of random, intermittent, loud pure tones (100 dB, 3000 Hz, maximum duration of 7 seconds) delivered to subjects through headphones. In addition, intermittent mild shocks were administered through leads placed on the subject's forearm (2.5 mA, maximum duration of 7 seconds). A button with two lights (one green and one red) was positioned within reach of each subject's hand. The green light was labeled correct and the red light was labeled incorrect. Subjects in the controllable stress group were able to terminate the noise/shock stimuli with four depressions of the button, but only after a minimum exposure of 2 seconds. Subjects who successfully terminated the noise/shock before the end of the 7 second trial were signalled with the green light. Signalling from the red light at the end of the 7 second trail indicated to subjects that the noise/shock was automatically terminated.

Subjects

A total of 24 male subjects were recruited as participants in this study; however, two subjects dropped out of the study. One subject was dropped from the study because he missed his second session and was unable to reschedule the session within a week's time. Another subject asked to withdraw from the study upon administration of the stressor. Therefore, the final number of participants were twenty-two. Subjects were recruited by a newspaper advertisement asking for volunteers to participate in a study examining the effects of stress on health and task performance. Participants were between the ages of 21 and 36 (M = 27.68, SD = 4.49) years.

All subjects were in good physical health and had not taken any prescribed or non-prescribed medications in the month preceding their participation in the study. In addition, all subjects were non-smokers and moderate consumers of alcoholic beverages (averaging 0-7 drinks/week). Any person reporting a recent stressful life event (e.g., marital divorce or separation, recent death or serious illness of a loved one) was not allowed to participate in the study, and to reduce the risk of including subjects who had been exposed to potential pathogens, subjects were not considered eligible for the study if a member of their household was sick. In addition, all subjects were blood donors or had previous experience with catheterization procedures. Information regarding these criterion was obtained from subjects during a standardized telephone interview. Information read to subjects over the phone and questions asked during the interview are located in Appendix A (see Appendix A).

In order to assure that subjects were in good health, they were all given a physical examination by a physician prior to their participation in the study. This physical included an EKG and routine blood tests (e.g., SMAC-20; WBC with differential) to measure factors such as protein albumin, cholesterol, glucose, sodium, creatinine kinase, bilirubin, as well as percentages of lymphocytes, monocytes, and granulocytes (neutrophils, basophils, and eosinophils). Subjects were also screened using the Schedule for Affective Disorders and Schizophrenia (SADS; Endicott & Spitzer, 1978) prior to their participation in the study to ensure that none had a history of mental illness or alcoholism. Informed consent was obtained from all subjects, and they were paid $181.50 for their participation according to rates set by the normal volunteer office at the National Institutes of Health, Bethesda, MD. A copy of the consent form is included in Appendix A (see Appendix A).

Measures

Stress measures. Sources of stress that were independent of the stressor being studied were measured using the Schedule of Recent Events (SRE; Holmes & Rahe, 1967) and the revised Symptom Checklist (SCL-90R; Derogatis, 1977). The SRE was used to assess the number of life changes and stressful events that occurred during the six months prior to each subject's participation in the study. The SCL-90 provided a global index of distress by measuring anxiety, depression, anger, fear, and general somatic distress experienced by subjects during the two weeks prior to their participation in the study. Subjects also completed a background demographic questionnaire, a health survey, and questionnaire assessing their history of having blood drawn and their

overall anxiety about needles and phlebotomy procedures. The health survey, derived from the Cornell Medical Index, asked subjects to report on the frequency and severity of a range of illnesses and health problems, the duration of these illnesses, information about physician visits and treatments, and information about various habits (e.g., exercise, sleep, cigarette smoking, diet, and consumption of alcoholic and non-alcoholic beverages). The demographic questionnaire, the health survey, and the blood donor questionnaire are located in Appendix B (see Appendix B).

Mood was measured throughout the study using The Profile of Mood States (POMS; McNair, Lorr, & Droppelman, 1971). The POMS is a self-rated inventory consisting of 65 mood states scored from 0 (not at all) to 4 (extremely); the inventory includes subscales for anger, concentration difficulties, depression, fatigue, tension, and vigor. Using an analog scale, subjects were also asked to report on a number of additional feelings occurring throughout the study including the extent to which they felt frustrated, stressed, tense, happy, and angry. The stressfulness of the noise/shock stressor and the extent to which subjects felt control over the stressor was assessed using an analog scale as well. Questions included "How unpleasant was the noise/shock?". and "How helpless did you feel during the noise-shock condition?" Subjects were asked to make a mark on a line representing a continuum from "not at all" to "moderately" to "extremely." The stress analog questionnaire and the noise/shock questionnaire are included in Appendix C (see Appendix C).

Post-stressor performance was measured using an anagram task. The task consisted of 20 five-letter anagrams. Different letter patterns were used in control sessions than in experimental sessions (e.g., 2-4-5-1-3 vs. 4-2-1-5-3). Words such as "human" were presented in an anagram as "u-a-n-h-m" during the control session, and words such as "judge" were presented as "g-u-j-e-d" (scrambled in the other pattern) during experimental sessions. Performance was scored by dividing the number of anagrams solved correctly by the length of time required to solve each word. Previous studies using anagrams have shown performance deficits following exposure to uncontrollable noise (Gatchel & Proctor, 1976; Hiroto & Seligman, 1975).

Immunologic measures. A functional and a quantitative assay was used to measure immunocompetence and to track changes over the course of the study. Blood samples for immune assays were drawn at four time periods over the course of each session (see Figures 1 and 2). Lymphocyte responsiveness to mitogen challenge was measured because the measure is considered an *in vitro* model of immunocompetence and because the responsiveness of lymphocytes to PHA and Con A has been shown to be reduced in humans and in animals undergoing stress. Also, as discussed in the previous chapter, proliferation measures have shown rapid changes following acute stress. Numbers of T and B lymphocytes and a three part differential (lymphocytes, monocytes, and granulocytes) were also measured using flow cytometry. Cells were stained for flow cytometry using a method described by Hoffman, Kung, Hansen, and Goldstein (1980). Briefly, 100 μl samples of whole blood were incubated for 30 minutes at 4^{o}C with 20 μl of the following monoclonal antibodies (Beckton-Dickinson, Mountain View, CA): anti-Leu 1 (total T lympho-

cytes), anti-Leu 2 (suppressor/cytotoxic T-cells), anti-Leu 3 (helper/inducer T-cells), anti-Leu 12 (B cells), and mouse IgG (non-specific staining control). After the first incubation, samples were treated with 2 ml of ACK lysing buffer (NIH Media Unit, Bethesda, MD) for 3 minutes, washed and resuspended in fixative containing 1% paraformaldehyde. Lymphocyte subpopulations were analyzed using a fluorescein activated cell sorter (FACScan, Beckton Dickinson).

For proliferation assays, lymphocytes were separated under sterile conditions by Ficoll-Hypaque sedimentation and adjusted to a final concentration of 2×10^6 mononuclear cells per milliliter of RPMI 1640 medium with Hepes (Mediatech, Washington, D.C.) supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1mM sodium pyruvate, 0.1 mM nonessential amino acids, 5×10^{-5} M 2-mercaptoethanol, and 5% heat-inactivated fetal calf serum. PHA (phytohemagglutinin; Sigma Chemical Company, St. Louis, MO) was used at final concentrations of 25 and 50 μg/ml. Con A (concanavalin A; Sigma Chemical Company, St. Louis, MO) was used at final concentrations of 5 and 10 μg/ml. Background proliferation was measured by incubating cells in complete media only. Each assay was performed in triplicate. One-tenth of a milliliter of PHA/Con A was added to 2×10^5 lymphocytes (in 0.1 ml complete medium) in 96 well plates and incubated at $37^{\circ}C$ for a total of 3 days in a humidified incubator with 5% CO_2. After approximately 54 hours of incubation, the cultures were pulsed with 20 μl of tritiated thymidine (50 μCi/ml; ICN Pharmaceuticals, Inc., Irvine, CA) and allowed to incubate an additional sixteen hours before harvesting. Cells were harvested onto glass fiber filter paper using a PHD cell harvester (Cambridge Technology, Inc.). The incorporation of [^{3}H] thymidine into newly synthesized DNA in stimu-

lated and non-stimulated cultures was then determined with a Beckman LS5801 liquid scintillation counter.

Because immune function may be altered prior to the onset of symptoms associated with an underlying infection, subjects were asked to report whether or not they experienced any symptoms of a cold or the flu during the two week period after their participation in the study. Pre-stamped postcards were provided to subjects so that they could mail this information back to the experimenter to assure that they were not in the early stages of a cold or flu during their participation in the study. This measure was included so that altered immune function would not be confounded with illness.

Procedures

Twenty-four subjects were randomly assigned to one of four groups: subjects who would be exposed to a controllable stressor on day one of the study, subjects who would be exposed to a controllable stressor on day two of the study, subjects who would be exposed to an uncontrollable stressor on day one of the study, and subjects who would be exposed to an uncontrollable stressor on day two of the study. All subjects were told that the purpose of the experiment was to examine performance and physiological changes following exposure to different aversive stimuli, and that the experiment would be conducted in two parts, each lasting approximately three and a half hours and occurring on different days within a week's time period. All sessions began between the hours of 7:30 and 9:00 am, and subjects fasted on the morning of each session.

Baseline session. After providing a brief description of the study, informed consent was obtained, and subjects were seated in a quiet testing room where an intravenous catheter was inserted. The catheter was inserted in the subject's antecubital fossa and kept patent with a slow infusion of isotonic saline. An initial baseline blood sample was drawn at this time, and subjects were asked to complete the POMS and the stress analog scale. Subjects sat quietly for one hour after insertion of the catheter. After the one hour rest period, subjects spent the next 30 minutes filling out a background questionnaire, the health survey, the SCL-90, and the SRE. This time period for filling out questionnaires coincided with the time during which subjects were exposed to the noise/shock stressor in their experimental session. A second blood sample was collected after this 30 minute period.

In the third phase of the baseline session, subjects were administered 20 five-letter, single solution anagrams. These were presented one at a time on 3 x 5 index cards, and subjects were allowed a maximum of 60 seconds to solve each anagram. Prior to beginning this task, subjects were read the following set of instructions: "The next part of the experiment involves a test of aspects of your intelligence. You will be asked to solve some anagrams. As you may know, anagrams are words with the letters scrambled. The problem for you is to unscramble the letters so they form a word. The letters will be presented to you on index cards. When you've found the word, tell me what it is. Proper names and plural words are not acceptable. Most of the subjects we have tested have averaged 25 seconds per word but you will have a full 60 seconds per word. If you fail to give a correct answer after 60

seconds the next word will be presented. Now, there could be a pattern or principle by which to solve the anagrams, but that's up to you to figure out. I can't answer any questions now. Your final scores will be plotted with the scores of the other subjects tested."

At the completion of the anagram task, the third blood sample was drawn and subjects were asked to fill out another POMS and stress analog scale. Finally, subjects were asked to sit quietly for the remaining 90 minutes of the study, and during this time, they were allowed to read. After this 90 minute rest period, subjects were once again asked to complete the POMS and stress analog scale, and a final blood sample was collected. If subjects had already participated in the experimental half of the study, they were debriefed and asked to complete payment forms. All other subjects were reminded of their appointment for the second session of the experiment upon completing their baseline session.

Experimental session. The procedure used in this part of the study was a modification of procedures from previous studies examining uncontrollable stress in humans (Brier et al. 1987; Hiroto, 1974; Gatchel & Proctor, 1976). Procedures were identical for uncontrollable and controllable noise/shock subjects. If subjects had been randomly assigned to receive their experimental session on day one of the experiment, informed consent was obtained upon their arrival. As in baseline (non-stress) sessions, subjects were seated in the testing room where an intravenous catheter was inserted. After an initial blood sample was drawn, subjects were asked to complete the POMS and the stress analog scale. They remained seated and quiet for one hour before exposure to the noise/shock schedules. At this time, the following instruc-

tions were read to all subjects: "From time to time a loud noise and a moderately strong shock will come on for a while. This will create an unpleasant situation for you. Now, this is important so listen carefully. There is a solution to the problem and if you figure it out the noise and shock will stop. Therefore, the amount of unpleasantness you receive is dependent on your abilities to find the solution to the problem. It is up to you. There are two lights located on the box in front of you. The lights will tell you how each trial of noise and shock was controlled. If you find the way to stop the noise or shock, the green light marked 'CORRECT SOLUTION' will flash when the noise and shock stops automatically. Remember, when the green light flashes on this means you were successful, but if the red light flashes, this means you did not stop the noise and shock, but they stopped automatically. Taking off or adjusting the position of either the headphones or the shock electrodes is not the way to stop the noise and shock and you should not touch them. You should not disassemble the testing apparatus. During the course of the experiment, please remain seated and move as little as possible. Once again, there is a way to stop the noise and shock but it is your job to figure out how."

The total noise/shock stressor exposure time was 30 minutes. Controllable noise/shock subjects were able to terminate the stressor with four button presses, yet uncontrollable noise/shock subjects were not able to terminate the noise/shock, but instead they were administered an identical pattern of noise and shock from a previous subject who had been able to control the stressor. Following the stressor period, a second blood sample was drawn. After the sample was drawn, subjects completed the noise shock questionnaire which asked them to

rate the stressfulness of the noise and shock and to rate the extent to which they felt they could control the stressor. The remainder of the session was identical to the baseline session. Subjects were administered a set of anagrams, and at the completion of this task, another blood sample was drawn. Subjects were asked to complete the POMS and the stress analog scale after finishing the anagram task. During the remaining 90 minutes of the study, subjects sat quietly and were allowed to read. At the end of the final rest period, subjects were asked to fill out another POMS and stress analog scale and the final blood sample was collected. The catheter was removed, and if the subject had already participated in his baseline session, he was debriefed at this time.

During the debriefing period, subjects were asked whether they figured out the strategy for terminating the stressor. If subjects responded yes to this question, they were asked to report what the strategy was. After obtaining this information, the purpose of the study was described in detail, and subjects were allowed to ask any questions that they had about the study. Subjects were told that deception was used in the study in order to manipulate stressor controllability. It was emphasized to subjects who were unable to control the stressor that termination of the stressor was independent of their responses because of pre-planned computer programming designed to increase the aversiveness of the stressor. Subjects were informed that their inability to control the stressor was no fault of their own but that of the experimenter's. When all questions about the study were answered, subjects were thanked for their participation and given payment forms to complete. At this time, they were also given a

pre-stamped postcard to mail back to the experimenter with information regarding any illnesses that developed during the two-week period after their participation in the study.

Results

Overview

Baseline mood and immune data from the two groups were analyzed by t-tests in order to establish initial group comparability. Further analyses were aimed at determining whether exposure to the stressor evoked changes in mood, in anagram performance, and in immune function. In addition, whether stressor controllability mediated stress effects on these variables was analyzed. Repeated measures analyses of variance were used to assess changes in mood, anagram performance, and immune function over time across baseline and across stress conditions. Three factors were analyzed: stressor controllability, condition, and time.

Controllable or uncontrollable noise/shock stress was administered during experimental sessions; the noise/shock stressor was not administered during baseline sessions; and immune function was measured over four time points representing pre and post stressor periods: 60 minutes pre-stress (t = -60), immediately after the 30 minute noise/shock stressor (t = +30 minutes), upon completion of the 20 minute anagram task (t = +50 minutes), and after a 90 minute final rest period (t = +150 minutes; see Figures 1 and 2). Therefore, immune function was analyzed using a 2 x 2 x 4 factorial design with repeated measures on the last two factors: condition and time.

Three part differentials and percentages of lymphocyte subpopulations were calculated at the first and last time periods only. Mood was measured at three time points across each condition (t = -60, +50, and +150) while anagram performance was measured once during each

session immediately after the noise/shock stressor. The effectiveness of the stress manipulation was determined by examining mood and anagram performance data across baseline and experimental stress conditions. The effectiveness of the control manipulation was determined by examining responses to the noise/shock questionnaire that asked subjects to report the extent to which they felt control over the stressor. Although subjects were run through baseline and experimental sessions, the order of these conditions was counterbalanced. Therefore, order was a fourth factor analyzed in this experiment.

Group comparability

Initial analyses were directed at establishing comparability between groups. T-tests were run on background data, overall health data, and recent life stress data (scores from Schedule of Recent Events, SRE; Symptom Check List, SCL-90) to determine whether groups differed independent of the stressor manipulation. It was expected that subjects from both groups would not differ on these factors due to random assignment.

As predicted, SCL-90 scores did not differ between groups for concentration difficulties, interpersonal problems, depression, anxiety, anger, fear, suspicion, or alienation. However, subjects assigned to the uncontrollable stress group did report more somatic complaints (M = 2.00) coming into the study than subjects assigned to the controllable stress group (M = 0.64; $t(22) = -2.51$, $p<.05$). While these differences were statistically significant, they may not be meaningful considering the low scores in both groups. The highest number of somatic complaints possible was 48.

Although the SCL-90 was completed on each subject's baseline

day, half of all subjects had prior exposure to the stressor as a result of counterbalancing. To test whether prior exposure to uncontrollable stress was related to an increase in symptom reporting, a two way analysis of variance was performed on the number of somatic complaints crossing group with the order of stressor presentation. This analysis revealed that while there was a main effect for group $F(1,21) = 5.13$, $p < .05$, confirming the higher number of somatic complaints in subjects in the uncontrollable stress group, there was no main effect for order $F(1,21) = 1.54$, $p = .23$, suggesting that the higher number of somatic complaints were not the result of prior exposure to the uncontrollable noise/shock stressor. The analysis may not have revealed an effect, however, because it divided the group into such small sample sizes (approximately 5 subjects per group).

Number of somatic complaints were not correlated with any baseline dependent measure. However, the number of somatic complaints were related to post stress measures of immune function. Somatic complaints were positively related to lymphocyte responses to Con A at 5 μg/ml ($r = .75$, $p < .001$), to Con A at 10 μg/ml ($r = .58$, $p < .01$), to PHA at 25 μg/ml ($r = .59$, $p < .01$), to PHA at 50 μg/ml ($r = .58$, $p < .01$), and to percentages of monocytes ($r = .44$, $p < .05$). Significant correlations persisted even after exclusion of one outlier's data. Therefore, number of somatic complaints was included as a covariate in analyses of immune data.

Groups were comparable on demographic variables (e.g., education level, income, marital status), and no significant differences were found between groups for the number of recent life events reported on the SRE. Health histories of groups were also comparable. There were

no differences in the number of hospitalizations, doctor visits, or health problems reported over the last year. Groups did not differ in their histories of having blood drawn or in their overall anxiety about needles and phlebotomy procedures. Furthermore, groups were of comparable age (controllable stress subjects mean age = 27.18 years; uncontrollable stress subjects mean age = 28.18 years), height, and weight.

While subjects did not differ in sleep or exercise habits, there were differences in the number of subjects reporting that they never drank alcoholic beverages ($X^2 = 6.47,1, p< .01$). All subjects randomly assigned to receive uncontrollable stress were moderate drinkers (2-7 glasses of beer or wine a week) while nearly half of the subjects assigned to the controllable stress group reported that they never consumed alcoholic beverages. These differences may be insignificant in light of the fact that heavy consumers of alcoholic beverages were not allowed to participate in this study. However, t-tests comparing drinkers with non-drinkers on all initial baseline dependent measures revealed no differences between the two groups. Drinkers of alcoholic beverages were not different from non-drinkers on any post stress immune measures as well.

T-tests were also performed comparing controllable and uncontrollable stress subjects on initial baseline measures of mood, anagram performance, and immune status as well as function to assure that both groups were comparable along these dependent variables under baseline conditions. These tests revealed that the two groups were comparable along all of these dependent measures; therefore, at baseline groups were not different in overall mood, and they did not differ in their ability to solve anagrams. In addition, groups were comparable with

respect to lymphocyte responses to Con A and PHA, and they did not differ in their percentages of lymphocytes, monocytes, and granulocytes as calculated by three part differentials or in their percentages of total T cells, T-helper cells, T-suppressor cells, or B cells.

Manipulation check

After establishing group comparability, analyses were aimed at assessing the effectiveness of the control manipulation and at determining whether subjects regarded the noise and electric shock as stressful. All subjects were asked to complete a questionnaire evaluating the noise/shock stressor and reporting the extent to which they felt control over the stressor. Table 2 contains data from this noise/shock questionnaire. Subjects with control did report having greater control (M = 10.3) than subjects without control (M = 7.3), and subjects with control reported feeling less helplessness (M = 4.2) than subjects without control (M = 6.3), yet neither of these differences reached statistical significance. Subjects with control over the stressor did, however, report feeling significantly more successful during the noise/shock condition than subjects without control ($t(20) = 8.05$, $p<.001$). Success scores averaged 12.54 when subjects had control over the stressor while success scores averaged 2.18 when subjects were unable to control the stressor. Data from the noise/shock questionnaire are summarized in Table 2.

Insert Table 2 About here

An additional finding was that subjects with control over the stressor reported the shock to be significantly stronger than subjects without control over the stressor ($t(20) = 9.88$, $p<.001$). Thus, subjects who were able to control the stressor felt more successful, but they also felt that the shocks were stronger ($M = 12.36$) than subjects who were unable to control the stressor ($M = 1.27$). The noise was rated to be slightly louder by subjects without control, but these differences were not significant. Overall, the control manipulation did seem to be effective in that subjects with control reported being more successful in the noise/shock condition than subjects without control. Although differences did not reach statistical significance for feelings of control and for feelings of helplessness, mean scores were in the predicted direction thereby suggesting that the control manipulation was effective (see Table 2).

Mood

Stressor controllability was an important factor directing changes in mood in response to the stressor. To examine differences between and within groups over time, repeated measures analyses of variance were performed on separate items from the stress analog questionnaire (e.g., anger, happiness, frustration, tension, and stress) and on subscales of the POMS (concentration difficulties, depression, and fatigue). Tukey post-hoc analyses were also conducted to determine differences among the mean mood scores.

Stress scores are illustrated in Figure 3. Analyses of stress scores revealed a main effect for condition $F(1,20) = 8.47$, $p<.01$. Overall, stress scores were higher during the experimental session than they were during the baseline session. Analyses also revealed a main

effect for time $F(2,40) = 11.98$, $p<.001$ and an interaction between time and condition $F(2,40) = 9.93$, $p<.001$. This interaction was qualified by a three-way interaction between condition, time, and group $F(2,40) = 3.45$, $p<.05$. This three-way interaction was due to the fact that stress scores from subjects exposed to uncontrollable stress increased with time during the stress condition but not during the baseline condition. This pattern did not occur in subjects exposed to controllable stress (see Figure 3). Mean comparisons indicated that at 50 minutes post-stressor initiation, stress scores were significantly higher in subjects without control than they were at the corresponding time period from their baseline day ($p<.01$). Subjects with control over the stressor did not show significant changes in reported stress during either baseline or stress conditions.

In examining frustration scores, it was found that subjects with control over the stressor did not report greater frustration in response to the stressor. The analysis revealed main effects for time $F(2,40) = 4.63$, $p = .02$ and condition $F(1,20) = 12.44$, $p<.005$ which were qualified by an interaction between the two $F(2,40) = 11.06$, $p<.005$. Overall, subjects were more frustrated during their stress session than during their baseline session. However, there was a significant three-way condition by time by group interaction $F(2,40) = 6.31$, $p<.005$. The three-way interaction occurred because frustration scores increased in response to the stressor but only in subjects unable to control the stressor. As illustrated in Figure 4, as long as subjects could control the stressor it caused little change in frustration scores; yet, when subjects were unable to control the stressor, they reported significantly increased frustration.

Analyses of anger scores paralleled those of frustration. Anger scores were marginally higher during the experimental condition $F(1,20) = 3.51$, $p = .08$. In addition, a condition by time interaction approached significance $F(2,40) = 3.06$, $p = .06$ indicating a tendency for anger scores to increase in response to the stressor. The analysis also yielded a condition by group interaction ($F(2,40) = 7.55$, $p = .01$) that was qualified by a three-way interaction ($F(2,40) = 7.59$, $p<.005$) between condition, time, and group. As shown in Figure 5, anger scores increased over time, yet as with frustration scores, the increase occurred only in subjects who were unable to control the stressor.

A three-way interaction was also revealed from the analysis of happiness scores $F(2,40) = 3.24$, $p<.05$. Self-reports of happiness decreased during the stressor, but only in subjects without control over the stressor and not in subjects with control over the stressor. These data illustrated in Figure 6. Mean comparisons revealed that subjects unable to control the stressor reported themselves to be significantly less happy immediately after the stressor ($p < .05$). Happiness scores also increased in this group by the end of the experimental session ($p < .05$) . Therefore, stressor controllability directed changes in anger, frustration, and happiness.

While subjects unable to control the stressor reported greater frustration, greater anger, and less happiness than subjects who could control the stressor, both groups reported greater tension after exposure to the noise/shock stressor. The repeated measures analysis of tension scores revealed a main effect for condition $F(1,20) = 8.47$, $p<.01$ and a main effect for time $F(2,40) = 11.98$, $p<.001$ which was qualified by an interaction between the two $F(2,40) = 9.93$, $p<.001$. As

illustrated in Figure 7, both groups reported greater tension during stress conditions than they did during baseline conditions, and scores returned to baseline levels at the end of the session.

Repeated measures analyses of variance were also run on data obtained from mood subscales of the POMS (e.g., depression, fatigue, and concentration difficulties). Analyses revealed that there were no changes in depression or fatigue scores for either groups. Analyses of concentration scores, however, yielded a significant three-way condition by time by group interaction $F(2,40) = 8.98$, $p<.05$. As illustrated in Figure 8, subjects unable to control the stressor reported greater difficulty concentrating immediately after exposure to the noise/shock stressor while subjects able to control the stressor reported less difficulty concentrating in response to the noise/shock. Tukey post-hoc analyses revealed that, overall, subjects without control reported greater difficulty concentrating than subjects with control immediately following exposure to the stressor ($p<.05$). These differences did not persist, as there were no group differences at the end of the stress session.

In summary, control mediated changes in mood occurring in response to the noise/shock stressor. Subjects exposed to uncontrollable stress became more angry, more frustrated, and less happy while subjects able to control the stressor did not show changes in these moods across stress sessions. In addition, stress scores were higher on the stress day than on the baseline day in subjects exposed to uncontrollable stress but not in subjects exposed to controllable stress. Groups did not differ in self-reported fatigue or depression, but they did differ in concentration abilities following the noise/shock stressor.

Subjects without control reported greater difficulty concentrating after the stressor while subjects with control reported less difficulty concentrating. While both groups reported increased tension following exposure to the stressor, overall mood changes reflected differences between having control and lacking control over the stressor.

Anagram performance

Anagram data were analyzed to determine behavioral aftereffects of the stressor experience. Performance was measured by dividing the number of anagrams solved by the total time spent on the task. Repeated measures analysis of variance of performance scores yielded no main effects. Figure 9 shows anagram performance of both groups on baseline and on stress days. Overall, both groups performed equally well during baseline and after stress conditions. Although subjects with control scored slightly higher on the anagram task in both conditions than did subjects without control, these differences were not significant.

Because subjects tended to perform better on the anagram task with time, performance scores were analyzed using a repeated measures analyses of variance from day one of the study to day two of the study to examine group differences in this "practice" effect. Order was also factored into the analyses to determine whether exposure to the stressor on day one of the study as opposed to exposure on the second day of the study had an effect on performance increases due to practice. The analyses revealed a main effect of time $F(1,18) = 9.02$, $p<.01$ and a group by time interaction $F(1,18) = 6.00$, $p<..05$. This interaction was due to an increase in anagram performance from day one of the study to day two of the study that occurred in subjects who were able to control the stressor. As illustrated in Figure 10, subjects unable to control

the stressor did not perform better on the anagrams during their second session while subjects who were able to control the stressor did. Whether the stressor occurred during the first session or during the second session of the study was an independent factor in this relationship. Therefore, exposure to uncontrollable stress appeared to impair the tendency to improve in anagram performance with practice.

Immune data

Immune data were analyzed in the same manner as self-report and behavioral data. A repeated measures analyses of variance was used to examine immune changes between groups over time and across conditions. Baseline measures were used as covariates in these analyses because it was anticipated that changes over time would be related to initial baseline values. Because two subjects reported that they experienced symptoms of a cold during the two week period following their participation in the study, analyses were preformed on data from all subjects and on data excluding the two subjects who reported symptoms of a cold following their participation in the study. One subject was represented from each group, and both subjects showed immune measures that were within normal ranges. The results of both sets of analyses were comparable; therefore, analyses of the larger sample are reported here.

Immune function. Figures 11 and 12 illustrate lymphocyte responsivity to Con A at 5 μg/ml and 10 μg/ml respectively. For Con A at 5 μg/ml, there was a significant main effect of time $F(3,54) = 3.63$, $p<.02$ qualified by an interaction between time and group $F(3.54) = 5.68$, $p<.01$, an interaction between condition and group $F(1,17) = 7.51$, $p<..02$, and a three-way interaction occurring between all three factors $F(3,54) = 4.75$, $p=.005$. The significant three-way interaction

was due largely to the fact that only subjects who were able to control the stressor exhibited a reduction in lymphocyte function, while subjects unable to control the stressor did not exhibit altered lymphocyte function. Therefore, controllable stress caused a reduction in lymphocyte responsivity, while uncontrollable stress did not. Uncontrollable stress resulted in an increase in lymphocyte responsivity to Con A at 5 μg/ml, but this increase did not reach statistical significance. Mean comparisons revealed that subjects who were able to control the stressor exhibited reduced lymphocyte function from baseline 50 ($p<.05$) and 150 minutes ($p<.01$) after initiation of the stressor. In addition, at the end of the stress session subjects without control had higher lymphocyte responses than subjects with control ($p<.01$).

Lymphocyte responsivity to Con A at 10 μg/ml followed a similar pattern to responsivity at the lower dose of Con A (see Figure 12). However, while there was a main effect for time $F(3,54) = 3.84$ and an interaction between time and group $F(3,54) = 2.99$, $p<.05$, group differences only approached significance during stress conditions $F(1,17) = 3.87$, $p=.066$. Lymphocyte responses to Con A at 10 μg/ml tended to be lower during the stress session in subjects who were able to control the stressor than in subjects who were unable to control the stressor, but the effect was not as strong as it was at the lower dose of Con A.

Figures 13 and 14 illustrate lymphocyte responsivity to PHA at 25 μg/ml and 50 μg/ml respectively. The analysis of responses to PHA at the lower dose revealed a significant time by group interaction $F(3,54) = 3.16$, $p<.05$). Once again, while the pattern of responses mirrored the pattern of responses to the lower dose of Con A (see Fig-

ure 13), group differences across conditions merely approached significance $F(1.17) = 3.10$, $p=.096$. Therefore, groups differed over time, but during stress conditions, these differences were marginal.

Responses to the higher dose of PHA also held a similar pattern. Analyses revealed a significant interaction between condition and group $F(1,17) = 4.66$, $p<.05$ as well as an interaction between time and group $F(3,54) = 3.39$, $p<.05$. Tukey post-hoc analyses of group means across conditions did not reveal significant differences, yet mean lymphocyte responses to PHA (50 μg/ml) were generally higher during stress conditions in subjects exposed to controllable stress. However, subjects in the controllable stress group also exhibited a different pattern of immune change over time than did subjects in the uncontrollable stress group, and these differences occurred during baseline conditions as well as during stress conditions.

While there were slight differences in lymphocyte responses to Con A and PHA, and to the different doses within each mitogen, lymphocyte responses to Con A and PHA at both doses held very similar patterns. In order to test the relatedness of lymphocyte responses to the two mitogens, correlations were run among post-stress lymphocyte responses to Con A (5 and 10 μg/ml) and PHA (25 and 50 μg/ml). Results revealed that responses to Con A and PHA were all highly interrelated. For example, the lower dose of Con A was related to the higher dose of Con A ($r = .84$, $p < .001$) as well as to the low and high doses of PHA ($r = .61$, $p < .001$ and $r = .58$, $p < .01$ respectively). The higher dose of Con A was also related to low and high doses of PHA ($r = .74$, $p < .001$ and $r = .76$, $p < .001$ respectively). Low and high doses of PHA were highly correlated within each other as well ($r = .95$,

$p < .001$). These correlations are summarized in Table 3.

Insert Table 3 about here

Immune Status. Figures 15 and 16 show changes in the percentages of T-helper cells (anti-Leu 3) and B cells (anti-Leu 12) respectively. Analyses of lymphocyte subpopulation data revealed that there were no main effects for percentages of T-lymphocytes (anti-Leu 1) or for percentages of T-suppressor cells (anti-Leu 2). However, the analysis of T-helper cell data revealed a three-way interaction between time, group, and condition that approached significance $F(1,14) = 4.36$, $p = .055$. During stress sessions, percentages of T-helper cells tended to decline in subjects with control over the stressor while percentages of T-helper cells tended to increase in subjects who were unable to control the stressor (see Figure 15).

The analysis of B cell data revealed a main effect for time $F(1,11) = 15.8$. Both groups exhibited increases in percentages of B cells during baseline and during stress conditions. The analysis also yielded an interaction between group and time $F(1,12) = 5.22$, $p<.05$ indicating that subjects in the uncontrollable stress group exhibited greater increases in percentages of B cells over time than subjects in the controllable stress group regardless of condition (see Figure 16). While percentages of B cells tended to be higher in both groups during stress condition, this difference was only marginally significant $F(1,12) = 3.43$, $p=.09$. Stress effects may have been minimized by counterbalancing procedures which required half of the subjects to participate in baseline sessions a few days after being exposed to the stres-

sor. If percentages of B cells rose as a response to uncontrollable stress and this effect was long lasting, it is possible that a "carry over" stress effect may have caused the overall pattern of observed differences. However, when order was factored into the analysis, a main effect was not revealed $F(1,9) = 0.86$, $p = .86$, although this may have been due to the small cell sizes ($n = 4$) that resulted from the analysis.

Data from three part differentials (measuring percentages of lymphocytes, granulocytes, and monocytes) were collected at the first and last time points of each session. Repeated measures analyses of variance revealed that percentages of lymphocytes and granulocytes changed over time, but neither change occurred in response to the stressor. Percentages of granulocytes decreased over time during baseline conditions as well as during stress conditions for both groups of subjects $F(1,14) = 6.17$, $p<.05$. Percentages of lymphocytes, on the other hand, increased over time, although this increase was marginally significant $F(1,14) = 3.71$, $p<.075$.

Analyses of monocyte data yielded a significant condition by group interaction $F(1,13) = 8.57$, $p=.01$, which was qualified by a three-way condition by time by group interaction $F(1,14) = 6.64$, $p<.05$. These data are illustrated in Figure 17. While percentages of monocytes increased in response to the stressor in subjects without control, monocyte percentages decreased in response to the stressor in subjects who were unable to control the stressor. Thus, the stressor had differential effects on monocyte percentages as a function of its controllability. Mean comparisons revealed that at the last time point of the stress session (t=+150), percentages of monocytes were signifi-

cantly higher in subjects who were unable to exert behavioral control over the stressor than in subjects who could control the stressor ($p<.01$; see Figure 17).

Relationships between mood, control, and immune states

In order to more closely examine the relationship between mood and immune function, correlations were run between mood scores collected immediately after the stressor and lymphocyte responses to Con A and PHA measured at the end of the experimental session. These time points were chosen because they represented the times during which greatest changes in mood and immune function were observed. Lymphocyte responses to Con A at 5 μg/ml were positively related to scores for tension ($r = .42$, $p < .05$), frustration ($r = .45$, $p < .05$), stress ($r = .36$, $p = .05$), anger ($r = .40$, $p < .05$), and for difficulty concentrating ($r = .42$, $p < .05$). In general, negative affect was related to higher immune function as measured by lymphocyte responses to Con A at 5 μg/ml only. Therefore, the hypothesis that immune function would be inversely related to negative mood was not supported, and for lymphocyte responses to the lower dose of Con A, a reverse relationship was revealed.

To test whether subject's perception of control and success were related to immune function, correlations were run between scores obtained from the noise/shock stressor questionnaire and immune measures obtained at the last time point of the stress session. While control scores were marginally correlated with percentages of monocytes ($r = .36$, $p < .08$), feelings of control over the stressor were not related to any other immune measures. However, self-reported success was inversely related to lymphocyte responses to Con A at 5 μg/ml ($r =$

-.52, $p < .01$) and at 10 μg/ml ($r = -.49$, $p = .01$). Hence, greater feelings of success were related to lower lymphocyte function. In addition, the stronger a subject reported the shocks to be, the lower the lymphocyte responses were to Con A at both doses ($r = -.42$, $p < .01$) and ($r = -.37$, $p < .05$ respectively). The unpleasantness of the noise/shock stressor was also related to lower lymphocyte response to the loser dose of Con A ($r = .64$, $p < .01$). Overall, feelings of unpleasantness were related to lower lymphocyte responses to Con A, and feelings that the shock was strong were related to lower lymphocyte function and lower percentages of monocytes. In order to investigate whether these relationships existed within groups, correlations were run within each group separately. Relationships were not seen when correlations were run within each group, but this may have been due to small sample sizes and the restricted ranges of scores within groups.

Because subjects with control over the stressor tended to make more button presses than subjects unable to control the stressor, group comparisons were made on this variable. When groups were compared for overall effort during the stress condition (number of times subject pushed the button), the analysis revealed that subjects with control over the stressor made more button presses than subjects without control $t(19) = 9.73$, $p < .001$. On the average, subjects with control pressed the button 218 times while subjects without control averaged a total of 73 responses. To determine whether response rates predicted immune function at the end of the experimental session, correlations were performed between effort and immune measures. Effort was highly correlated with lymphocyte responses to Con a at the lower dose ($r = -.51$, $p < .01$), but not with responses to the higher dose of Con A,

responses to PHA, or percentages of monocytes. In order to examine whether effort was related to immune measures within groups, correlations were run between the number of button presses and immune measures within groups separately. These correlations yielded comparable coefficients between groups.

Discussion

The purpose of this study was to examine whether an acute stressor would elicit a change in aspects of human immune function. Also examined was whether behavioral control over the stressor would act as a mediator of stress-effects on immunologic states. It was hypothesized that exposure to a brief stressor would lower lymphocyte function, and furthermore, that lymphocyte function would be lowered to a greater degree if the stressor were uncontrollable as opposed to controllable. It was also predicted that subjects without control would report greater stress and exhibit poorer task performance when compared to subjects with control.

Interestingly, while the data from this study suggest that acute stress-effects on aspects of immunologic function may be mediated by control, results were not as predicted. Original hypotheses were that uncontrollable stress would lead to lower immune function than controllable stress. However, subjects exposed to uncontrollable stress did not exhibit altered immunologic function or status. In fact, subjects exposed to uncontrollable stress exhibited a slight elevation in lymphocyte function and in percentages of monocytes. Although these increases from baseline did not reach statistical significance, they did contribute to overall group differences in immune states that were present after exposure to the stressor.

While immune states were not significantly altered by exposure to uncontrollable stress, mood and task performance were. Subjects exposed to uncontrollable stress reported increased anger, tension, frustration, and decreased happiness compared to baseline measures. In

addition, subjects who received uncontrollable stress did not exhibit improved anagram performance with practice as did subjects who received controllable stress. Therefore, the uncontrollable stressor did have predicted effects on mood and behavior, but it did not have predicted effects on immunologic function.

When subjects exposed to the controllable stressor were compared with subjects who were unable to control the stressor, results indicated that these subjects exhibited a very different pattern of responses to the stressor. For example, subjects who could control the stressor exhibited a significant reduction in lymphocyte responsivity to Con A and in monocyte percentages ninety minutes after the stressor was terminated. Group differences were present at the end of the stress session despite comparable baseline immune states. Furthermore, only immune measures were affected by the controllable stressor. Subjects did not exhibit changes in mood or in anagram performance when they were able to control the stressor. Therefore, results suggest differential effects of controllable and uncontrollable stress on certain immune states in humans. Furthermore, data from both groups of subjects indicate that human immunologic states may be disassociated from mood states and from task performance.

The results of this study are surprising in light of data indicating that lymphocyte function is suppressed in rats following exposure in uncontrollable stress, but not controllable stress (Laudenslager et al., 1983). While it is difficult to compare results of animal studies to those of human studies, there are a number of explanations which could account for the results reported here.

First, rats may not be appropriate models for predicting the relationship between stress and immune function in humans because they may not respond immunologically during stress as humans do. Some argue, for example, that rat lymphocytes are more sensitive to corticosteroids than human lymphocytes (Claman, 1972), and while all do not agree with this position (Cohen & Crnic, 1982), it is possible that rats may not be sufficient models for studying the relationship between stress and human immune function because their stress hormones regulate immunocytes differently.

Another possible explanation why results of this study did not confirm those of Laudenslager et al. (1983) may be that there were time frame differences between the studies. For example, Laudenslager et al. (1983) collected blood samples from the rats twenty-four hours after animals were exposed to the stressor. Therefore, the animals were exposed to either controllable or uncontrollable stress, but blood was not sampled for immune measures until the following day, after the animals were briefly re-exposed to the stressor. The delay in sampling was intended to maximize differences between groups because it was thought that the immediate effects of the stressor on both groups might mask differences that were the result of the control manipulation (Maier, Laudenslager, & Ryan, 1985). Had measures been taken from the rats immediately after the stressor, results may have paralleled the results of this study. Similarly, had measures been collected from subjects in this study twenty-four hours after subject's completed their stress session, a reversal in the findings of this study may been observed.

For example, it is possible that immune function was reduced by

the uncontrollable stressor in this study, but that ample time was not provided to observe such an effect. Differences in immune function between the two groups were most significant at the end of the stress session. The session ended 150 minutes after the thirty minute stressor began. Therefore, it may be that early changes in immune function do not reflect long term changes, and that the direction of changes noted in this study may have been reversed hours after the final blood sample was collected. A number of studies report biphasic immune responses following exposure to stressful events (Brahmi et al., 1985; Jensen, 1969; Monjan & Collector, 1977). For example, Brahmi et al. (1985) report both increases and decreases in natural killer cell activity at different time points following an acute exercise stressor. Therefore, it is possible that a different pattern of immune responses may have been observed in this study had additional samples been collected a day later. The decrease in lymphocyte function exhibited by subjects exposed to controllable stress may have been followed by an increase in function twenty-four hours later. It is not known from the data presented here what the long term effects of the acute stressor were.

Although subjects in both groups received identical stressors in terms of intensity, pattern, and duration, there were differences between groups that were the product of their differential reinforcement schedules. For example, subjects who were exposed to the controllable stressor were signalled that their responses were correct more often than subjects who could not exert control over the stressor. As as result, subjects with control reported greater success than subjects without control. In this study, as in most

human learned helplessness studies, it is difficult to separate the effects of actual control from the effects of perceived success. This criticism has been reviewed by Winefield (1982). In this study, self-ratings of success were more highly related to lymphocyte function than were self-ratings of control. Therefore, some may argue, that exposure to controllable stress reduced lymphocyte function because it led to greater feelings of success rather than feelings of control.

A more likely explanation for lowered lymphocyte function in subjects who were signalled that their responses were correct might be that subjects receiving positive feedback simply responded with more button pressing than subjects who were signalled that their responses were incorrect. Analyses relating the number of button presses with immune function revealed that the two were inversely related. Therefore, the controllable noise/shock stressor may have caused a reduction in lymphocyte function because subjects with control were more active, and this heightened activity may have been responsible for driving immune responses down.

There is evidence supporting pathophysiological effects of behavioral control, particularly when response rates are high (Obrist et al., 1978; Solomon, Holmes, & McCaul, 1980; Weiss, 1968). For example, Obrist et al. (1978) has shown that avoidance responding, or "active coping," can result in increased arousal and, hence, suggests that the ability to exert behavioral control may not always reduce stress effects. It is possible that behavioral response demands in the face of stress may also lower lymphocyte function.

The effects of response rates, or of various schedules of rein-

forcement, on immune function have not been closely studied; nor have the differences between escape and avoidance schedules and their effects on immunity been established. Animal studies comparing stress effects of uncontrollable aversive events do not always report response rates regardless of whether avoidance or escape paradigms are used. For example, Laudenslager et al. (1983) do not report whether rats receiving uncontrollable stress made more responses to avoid the stressor than their yoked partners. Exposure to controllable stress may not have lowered lymphocyte function in these animals simply because they did not respond sufficiently to the stressor. In any event, the data may provide support to recent claims that there are "costs of coping" and that the absence of responding in the face of uncontrollable events may be adjustive (see Cohen, Evans, Stokols, & Krantz, 1986).

It is important that the data from this study are not interpreted as suggesting that acute, controllable stressors have negative effects on health because they lower certain aspects of immune function. While differences in immune states between groups reached statistical significance, it is difficult to understand fully the clinical relevance of these data. The data do not necessarily reflect altered susceptibility to disease following exposure to a controllable stressor. Only two subjects, one from each group, reported symptoms of a cold or flu following their participation in the study, and both had immune responses that were within clinically defined non-pathologic ranges. In fact, all subjects remained within non-pathologic ranges throughout the study. Because susceptibility to illness was not directly measured, it is difficult to draw conclusions

regarding the effects of the stressor on overall health.

To some extent animal studies have addressed questions concerning the clinical outcomes of exposure to controllable and uncontrollable stress by challenging animals with pathogens such as tumors. Tumor growth has been shown to be enhanced by uncontrollable stress and not by controllable stress in rats, but these effects are variable and can be reduced and even reversed following multiple stress sessions (Sklar & Anisman, 1979). Sklar and Anisman (1979) did not measure whether changes in tumor growth were related to changes in immune function. Shavit et al., (1983) report lowered lymphocyte responses and increased tumor growth in rats following stress, but more human research is needed examining the role of immunologic processes in the link between stress and disease (Kasl, 1983).

While the immunologic changes that were observed in this study may not be reflective of a clinical disorder or of decreased resistance to disease, this does not minimize the overall significance of these findings. To date there are few, if any, well-controlled human experiments offering empirical evidence of a causal relationship between stress and altered immune states, and the role of control as a potential mediator between stress and various aspects of immune function in humans has not been established. This study provides support for a causal relationship between acute stress and altered parameters of immune function and underscores the importance of control in directing this relationship.

Other studies investigating the relationship between stress and various aspects of immunologic function in humans have been unable to eliminate health-related behaviors as possible factors accounting for

altered immune function during the stressful life event (e.g., Arnetz et al., 1987; Bartrop et al., 1977; Kiecolt-Glaser et al., 1987; Kiecolt-Glaser et al., 1984). However, results of this study yielded changes in lymphocyte function and in percentages of monocytes within a two hour period while subjects remained seated in the laboratory. The implication of these findings is that chronic events are not necessary in order for a person to exhibit altered immune states, nor are changes in health-related behaviors such as diet, exercise, sleep, or consumption of alcohol.

In this study, immune changes were limited to lymphocyte proliferation to Con A and to percentages of monocytes. Lymphocyte responses to PHA showed similar changes in responses to controllable versus uncontrollable stress, but group differences during stress conditions only approached significance. Lymphocyte responses to Con A were highly correlated with responses to PHA, which may be explained, in part, by the fact that they are both T-cell mitogens. There is evidence, however, of differences between PHA and Con A. For example, PHA stimulates T-suppressor cells to a lesser degree than Con A (Reinhertz & Schlossman, 1980). In addition, lymphocytes that respond to Con A have been reported to be more dependent upon the presence of monocytes than PHA-stimulated lymphocytes (van Oers, Pinkster, & Zeijlemaker, 1979). Because percentages of monocytes were higher following exposure to controllable stress, this may explain why stronger stress effects were found for lymphocyte responses to Con A than to PHA.

Results yielding different responses between Con A and PHA stimulated lymphocytes are difficult to interpret. It is not clear whether both PHA and Con A provide measures of the same phenomenon or

whether they are highly correlated for other reasons. Many studies report reduced lymphocyte responses to both Con A and PHA during stress (Bartrop et al., 1977; Hedfors et al., 1976; Odio et al., 1986; Schleifer et al., 1983; Shavit et al., 1983), while others report significant results with PHA but not with Con A (Kiecolt-Glaser et al., 1987; Linn et al., 1984). Effects were stronger in this study for responses to Con A than they were for PHA. While concentrations of these mitogens differed across some of these studies, differences in results cannot be entirely explained by the various mitogen concentrations that were used. Issues are further complicated by evidence that various concentrations of PHA yield different responses at different time periods following stressful life events (Bartrop et al., 1977). Therefore, differences in lymphocyte responses within concentrations of Con A and PHA as well as between the two mitogens present complex issues that need to be resolved in order to interpret results of lymphocyte proliferation assays more clearly.

The results of this study were limited to lymphocyte responses to Con A and to percentages of monocytes and should not be generalized to be reflective of all facets of immune function. A number of immune measures were not tested in this study (e.g., natural killer cell activity, phagocytosis, antibody production etc.). Whether other aspects of immunologic function respond to acute stressors that are controllable or uncontrollable is not known. Other measures of immunocompetence might show different response patterns to controllable and uncontrollable stressors, as it is unlikely that all measures of immune function move unidirectionally in response to particular stressors. For example, Palmblad et al., (1976) found decreased phagocytosis during a

77-hour stress vigil in humans, yet at the same time, a simultaneous increase in interferon production was observed. Although controllable stress led to a decline in two immune parameters, other aspects of immune function may not have been affected by the stressor or may have been driven in other directions than those reported here.

In this study, various immune cells (e.g., B cells, granulocytes) were shown to change over time independent of exposure to the stressor. The importance of including appropriate control groups is illustrated by results such as these. For example, Landmann et al. (1984) report increased numbers of monocytes, natural killer cells, and B cells in response to an 8 minute cognitive stressor. Furthermore, they report increases in numbers of T-helper cells, T-supressor cells, and granulocytes in response to an exercise stressor. Control subjects were not studied nor were experimental subjects studied during non-stress session. Without the inclusion of a control group, and without studying the same subjects under comparable non-stress conditions, the results of the study are uninterpretable. Results reported by Landmann et al., (1984) may have occurred as a function of time and not of stress as was reported. Because so little is known regarding how immune function changes with time, from stress, or even due to laboratory procedures such as a blood sampling techniques, maintaining appropriate controls is critical. In addition, due to the great variability in immune measures between subjects, within subjects designs may be more meaningful.

In any case, this study provides information regarding the time frame in which immune changes can occur, and therefore offers a methodological framework for future research. Additional studies are needed

that examine how other immunologic responses change in response to controllable and uncontrollable stressors but more importantly what the ramifications are of changes in one parameter versus another. Immune measures should be collected across several hours, a day, or more to determine whether short-term stress effects reflect long-term status. Of equal importance would be studies investigating how chronic stress compares to acute stress.

It would also be interesting to determine whether acute stress reduction procedures might cause changes in immune function. Studies have reported changes in natural killer cell activity following months of relaxation therapy in a geriatric population (Kiecolt-Glaser et al., 1985), and increases in neutrophil function have been reported to occur following biofeedback training (Peavey, Lawlis, & Goven, 1985). In these studies, immune changes occurred after many sessions of relaxation training. Whether procedures such as these have the ability to impact immune processes acutely is not known.

The generality of these findings should also be established. The results of this study may be limited to young, healthy males. It would be interesting to extend these findings to other populations such as women, the elderly, smokers, patients with various medical illnesses, and psychiatric patient populations. Examining other factors such as personality and individual differences may also prove useful in future research. Clearly, all individuals do not respond in the same manner in response to "identical" stressors. It would be important to determine how various coping responses, for example, might have directed immune responses.

In conclusion, this study suggests that stressful stimuli can

bring about rapid changes in lymphocyte function and in percentages of monocytes, and that stressor controllability may serve as an important mediator directing this relationship. Studies are needed to further characterize immunological changes that occur as the result of exposure to stressful events, and the clinical relevance of these data must be determined. Finally, research is needed which collectively investigates the stressor, immune alterations, and their effects on subsequent susceptibility to disease before a relationship between stress and disease can be explained by stress-elicited changes within the immune system.

Table 1

Immunological Assays Used as Indices of Immunocompetence

I. Quantitative Indices of Immune Status
White Blood Count (WBC)
Differential Analysis (lymphocytes, monocytes, granulocytes)
Enumeration of Cell Populations and Sub-populations
Natural Killer Cells
B Cells
T Cells (e.g., T-helper cells, T-suppressor cells)
Antibody titers
II. Qualitative Indices of Immune Function
Lymphocyte Proliferation (Con A, PHA, PWM, PPD, LPS)
Mixed Lymphocyte Response (MLR)
Chemotaxis
Phagocytosis
Natural Killer Cell Activity
Lymphokine/Monokine Production (e.g., Interferon, IL-1, IL-2)
Generation of Specific Antibodies
Delayed Type Hypersensitivity (DTH)
Graft Versus Host Response

Table 2

Mean Responses to the Noise–Shock Questionnaire

(0 = not at all; 16 = extremely)

	Controllable Stress		Uncontrollable Stress
	Mean (SD)		Mean (SD)
How helpless did you feel during the noise–shock condition ?	4.18 (3.49)		6.27 (2.90)
How much control did you have over stopping the noise and shock?	10.27 (4.69)		7.27 (4.67)
How successful do you feel you were in the noise–shock condition ?	12.64 (2.62)	***	2.18 (3.37)
How strong did you think the shock was ?	12.36 (2.69)	***	1.27 (2.57)
How loud did you think the noise was ?	7.64 (2.46)		8.64 (2.94)

* $p < .05$
** $p < .01$
*** $p < .001$

Table 3

Intercorrelations Between Post-stress Lymphocyte Responses to Low and High Doses of Con A and PHA (n = 22)

	Con A 5 µg/ml	Con A 10 µg/ml	PHA 25 µg/ml	PHA 50 µg/ml
Con A 5 µg/ml	-----	.84 p < .001	.61 p < .001	.58 p < .01
Con A 10 µg/ml		-----	.74 p < .001	.76 p < .001
PHA 25 µg/ml			-----	.95 p < .001
PHA 50 µg/ml				-----

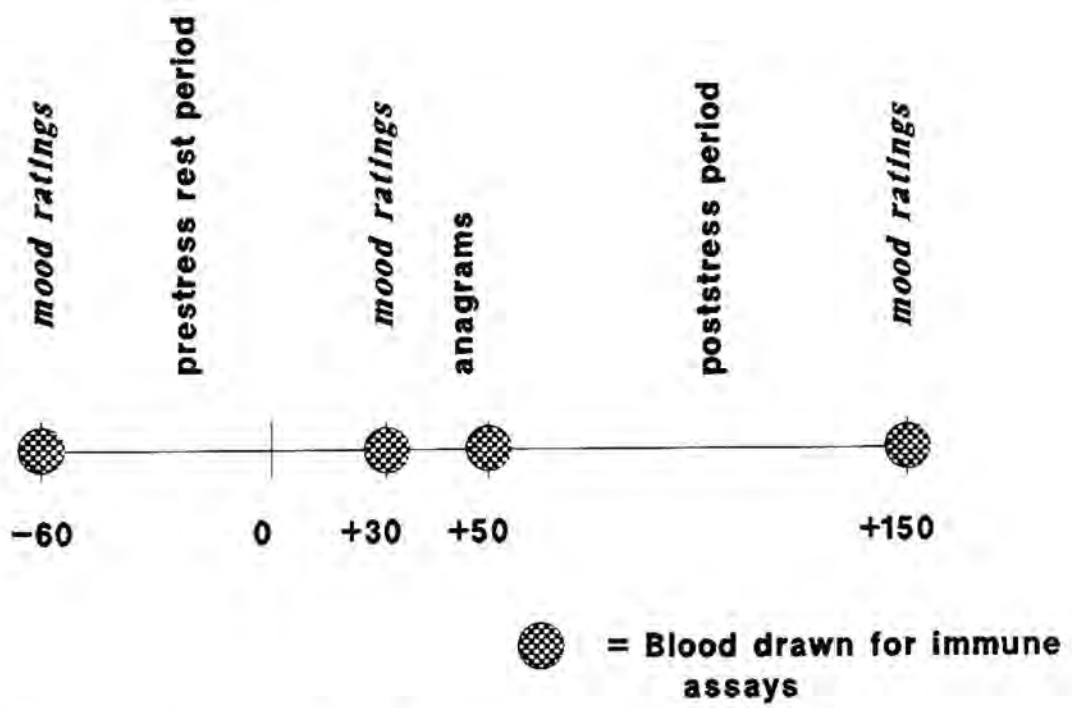

Figure 1. Timeline for baseline session

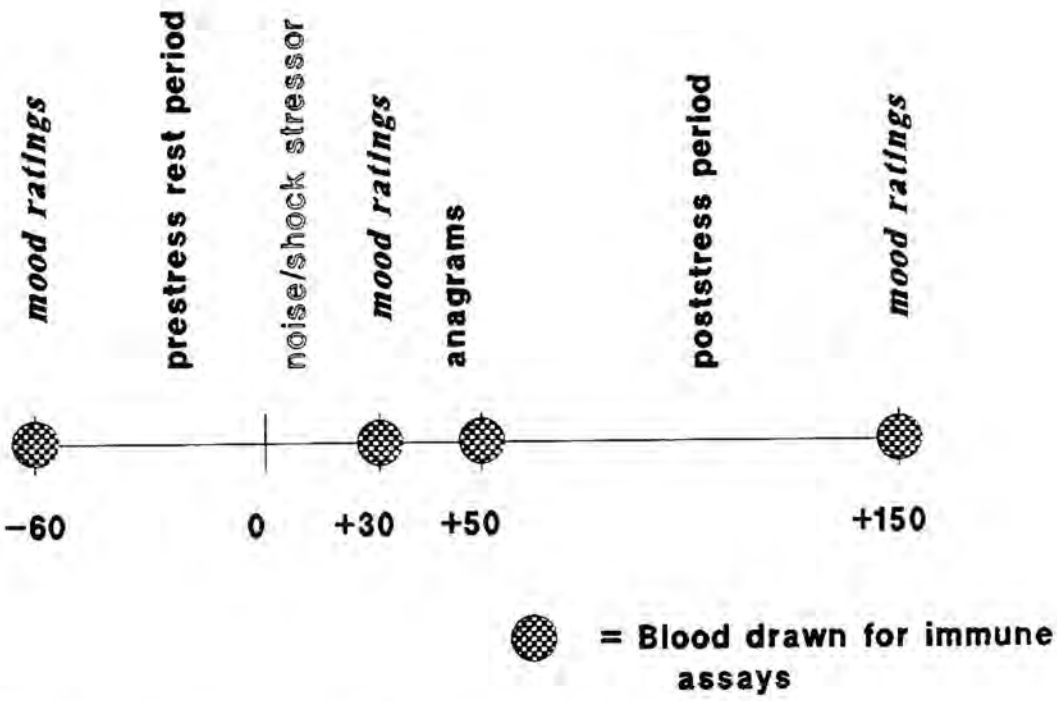

Figure 2. Timeline for experimental session

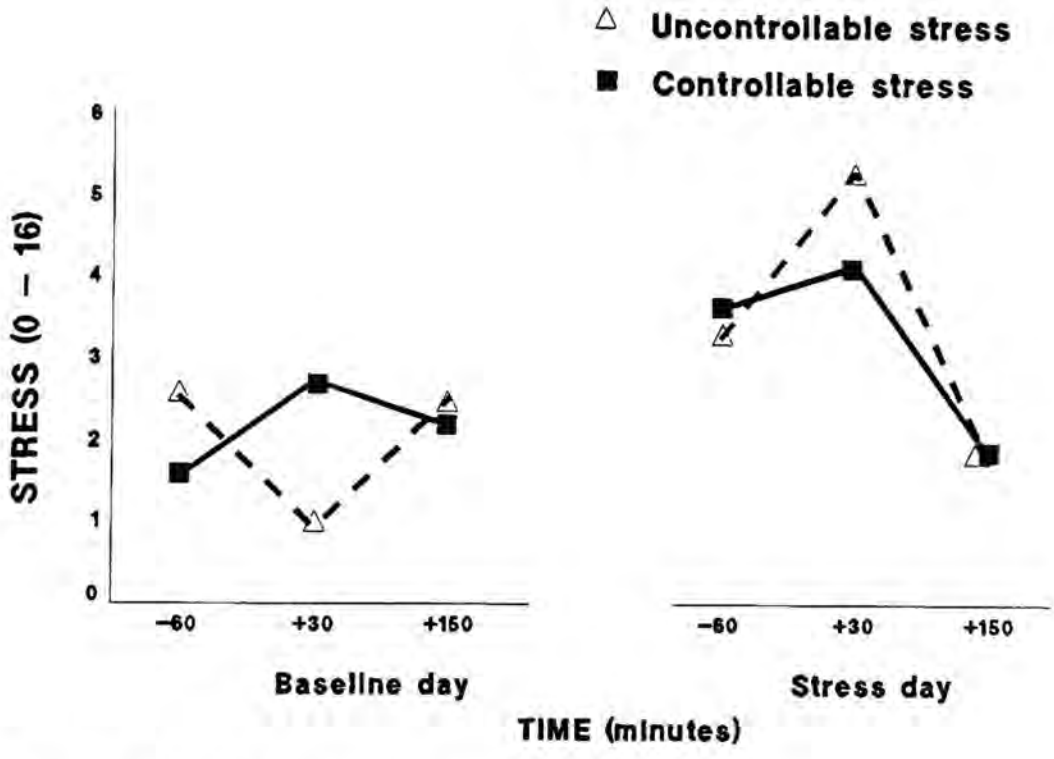

Figure 3. Mean stress scores across baseline and stress sessions

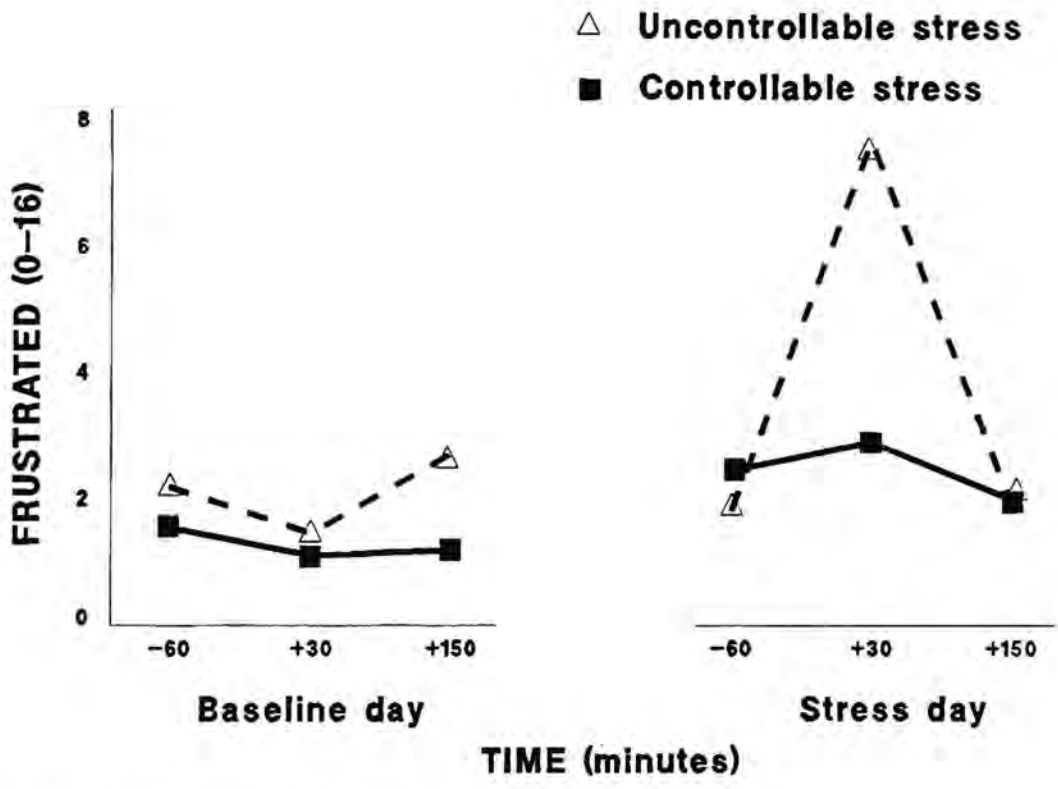

Figure 4. Mean frustration scores across baseline and stress sessions

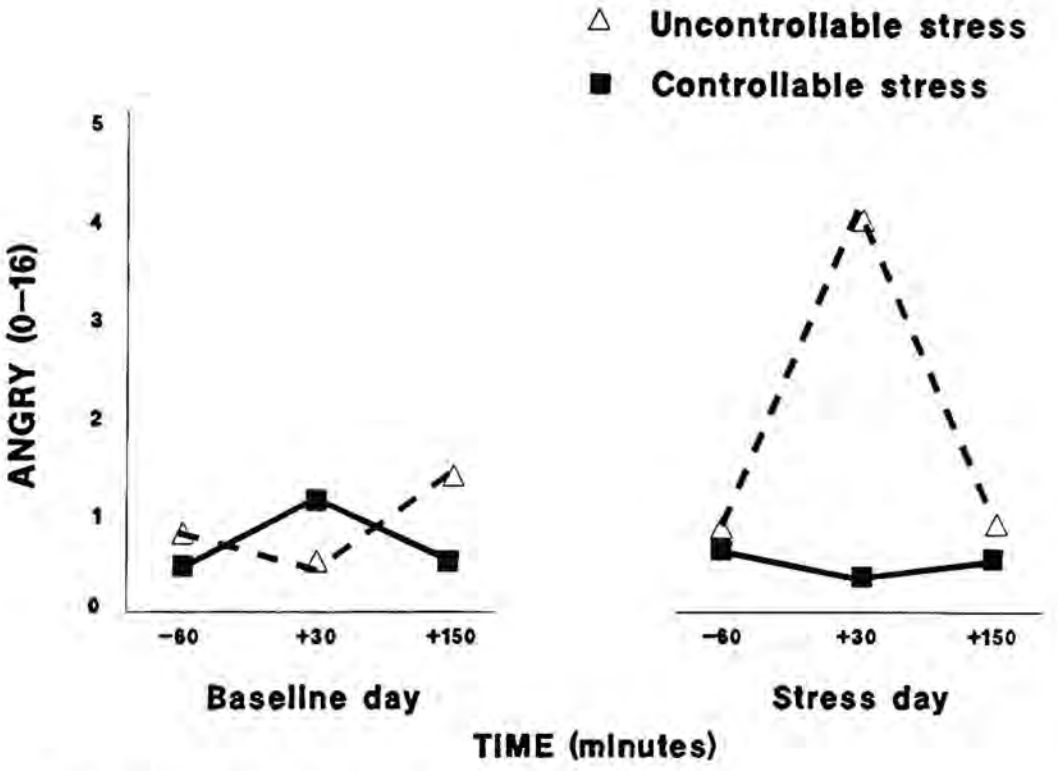

Figure 5. Mean anger scores across baseline and stress sessions

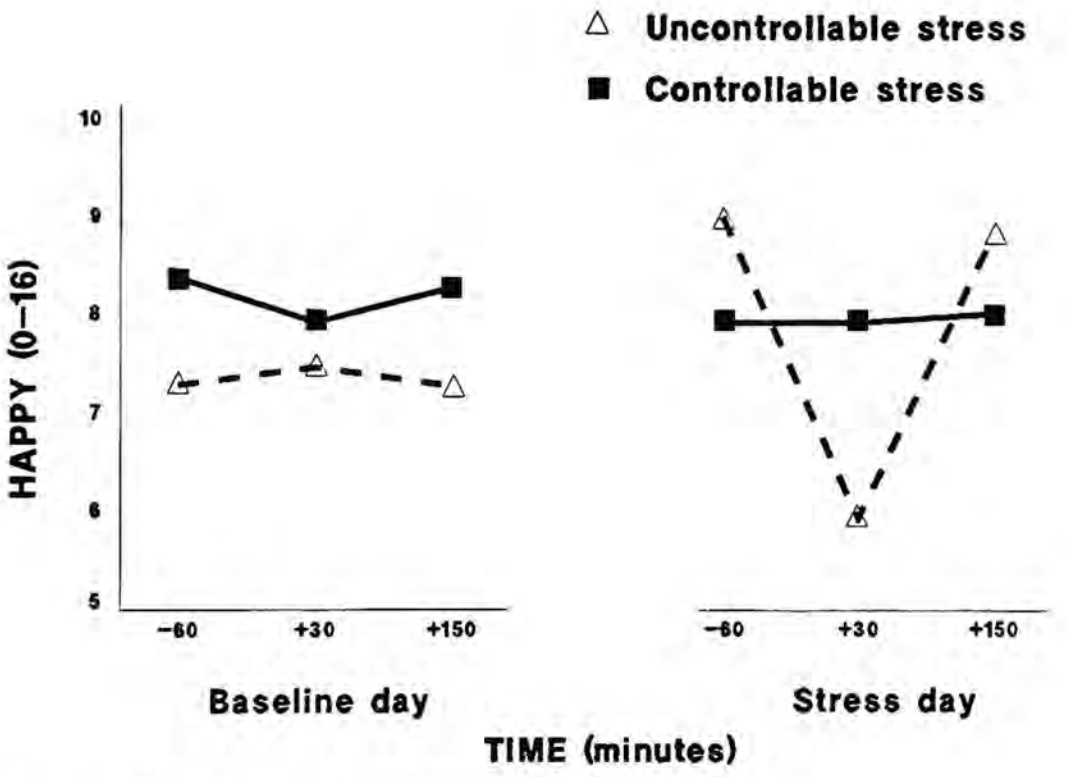

Figure 6. Mean happiness scores across baseline and stress sessions

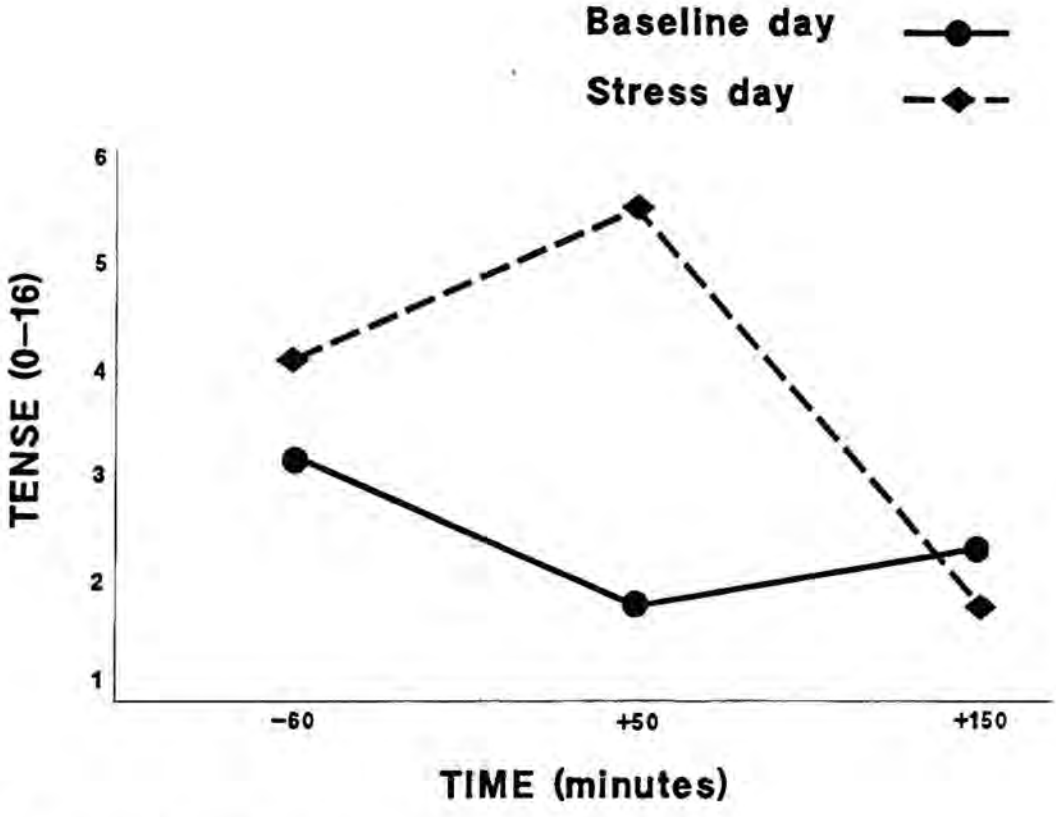

Figure 7. Mean tension scores across baseline and stress sessions

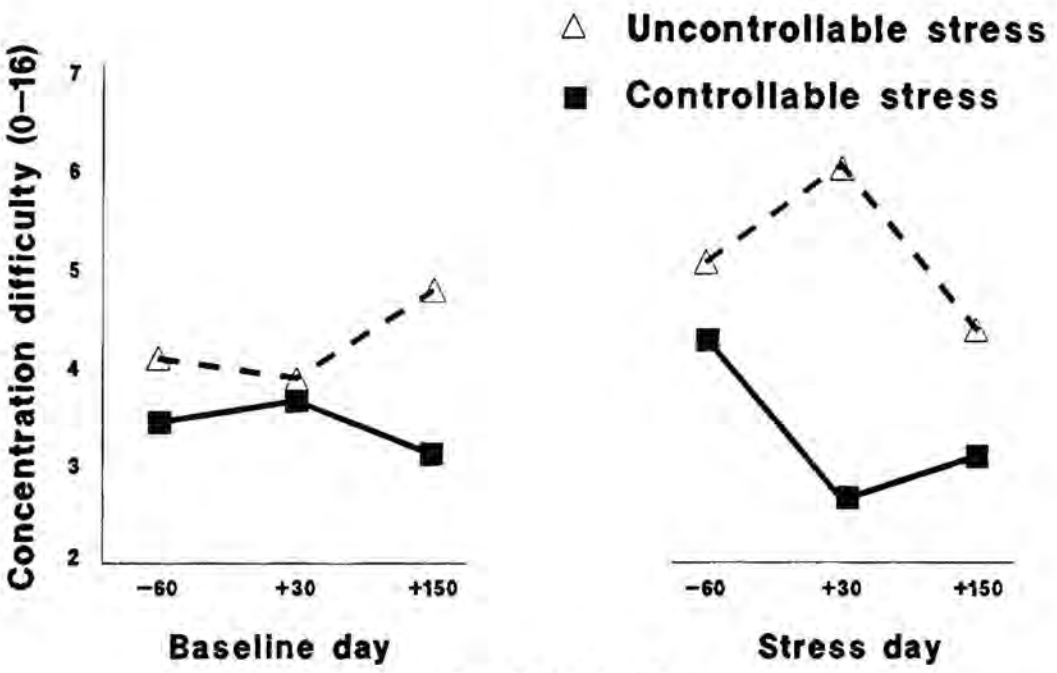

Figure 8. Mean scores of concentration difficulty across baseline and stress sessions

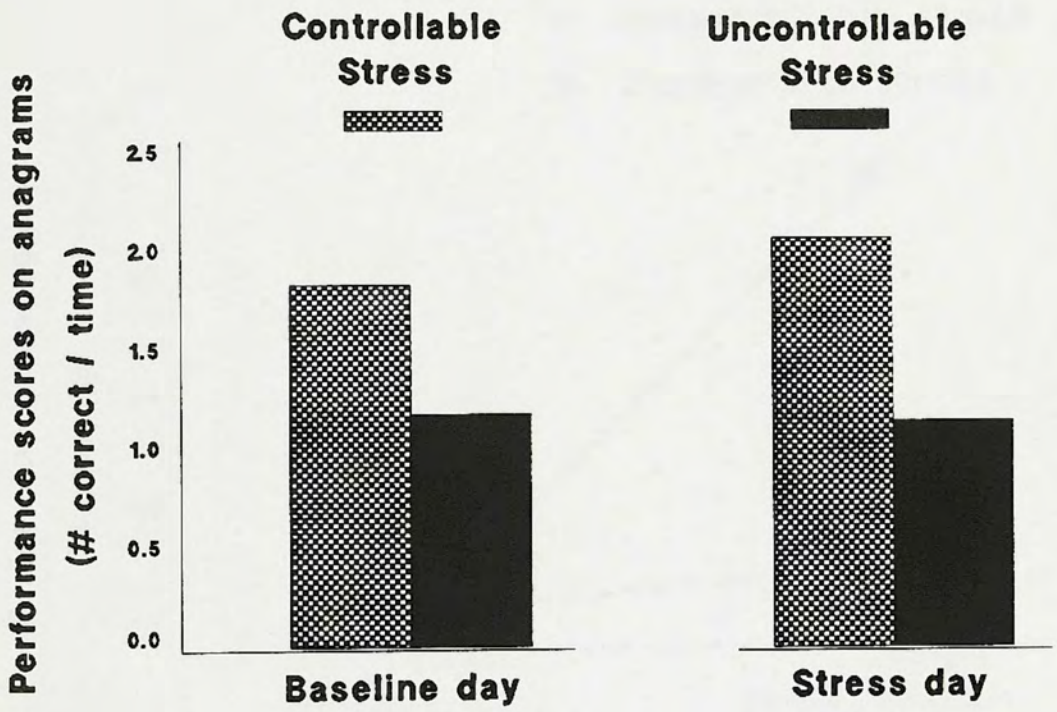

Figure 9. Mean anagram performance scores during baseline and stress sessions

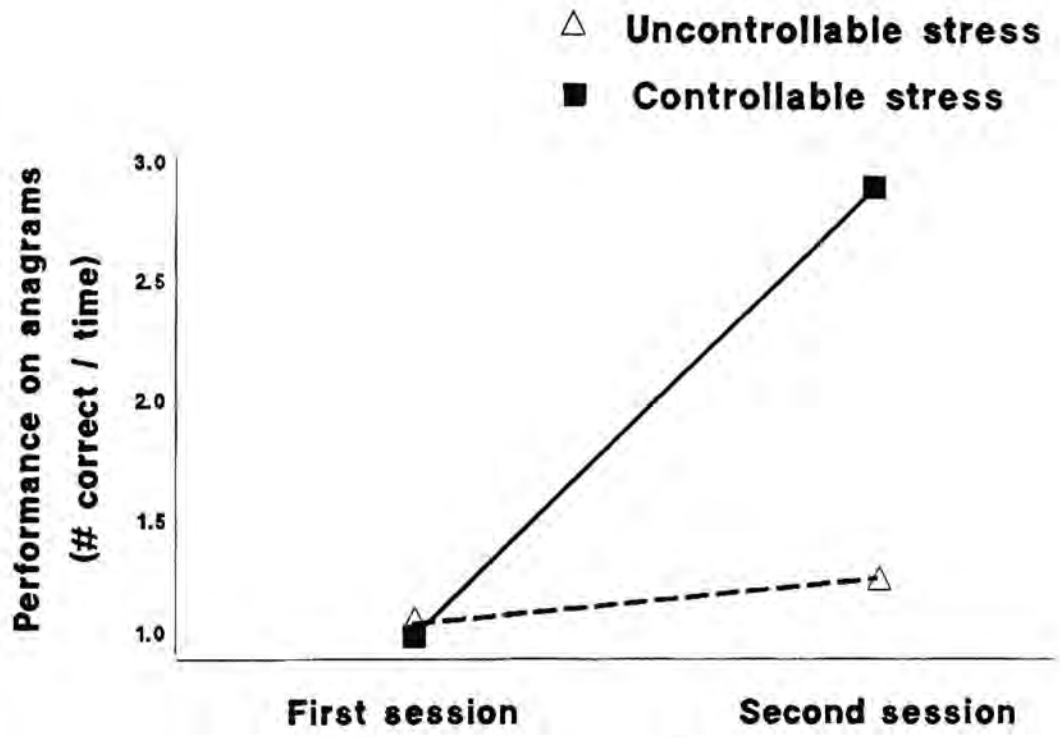

Figure 10. Improvement in anagram performance from session I to session II

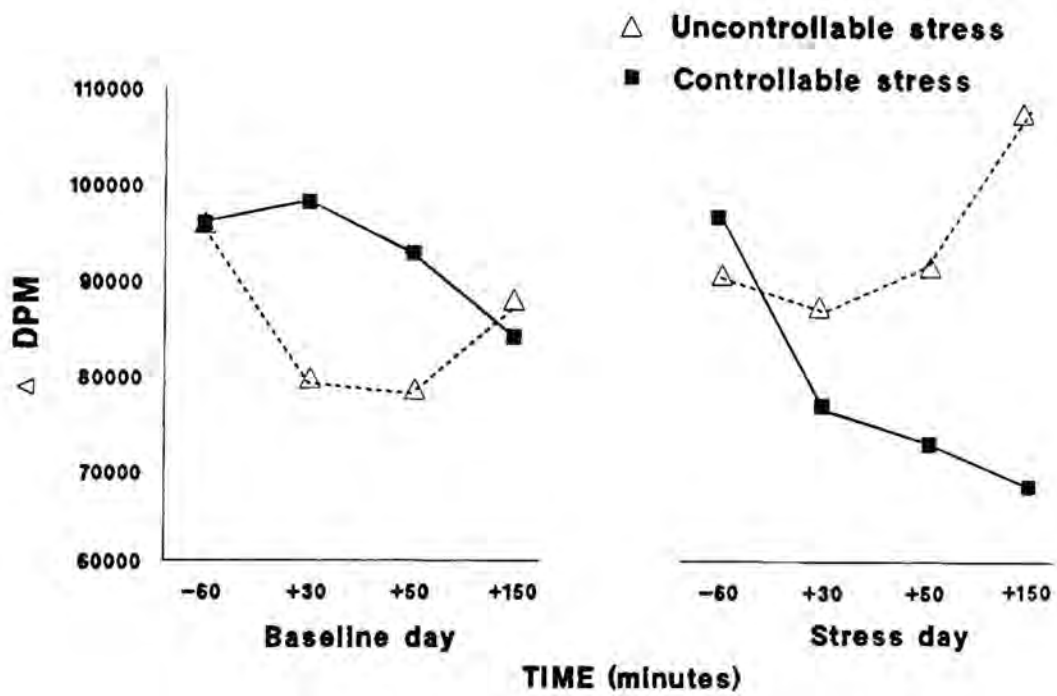

Figure 11. Lymphocyte proliferation to Con A (5 μg/ml) across baseline and stress sessions

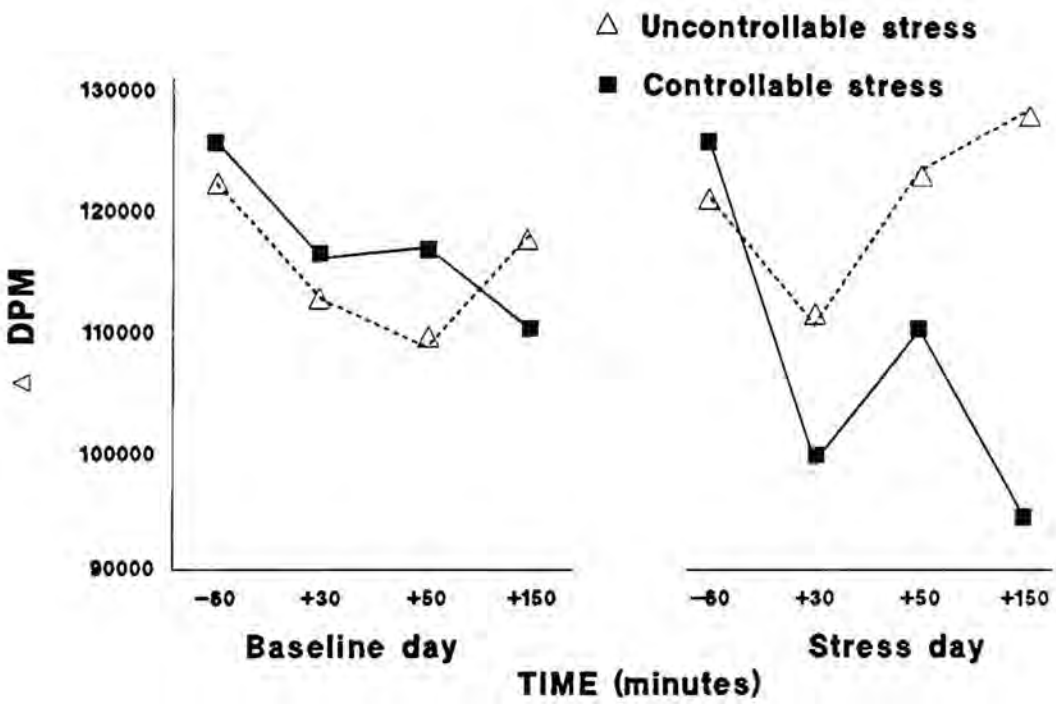

Figure 12. Lymphocyte proliferation to Con A (10 μg/ml) across baseline and stress sessions

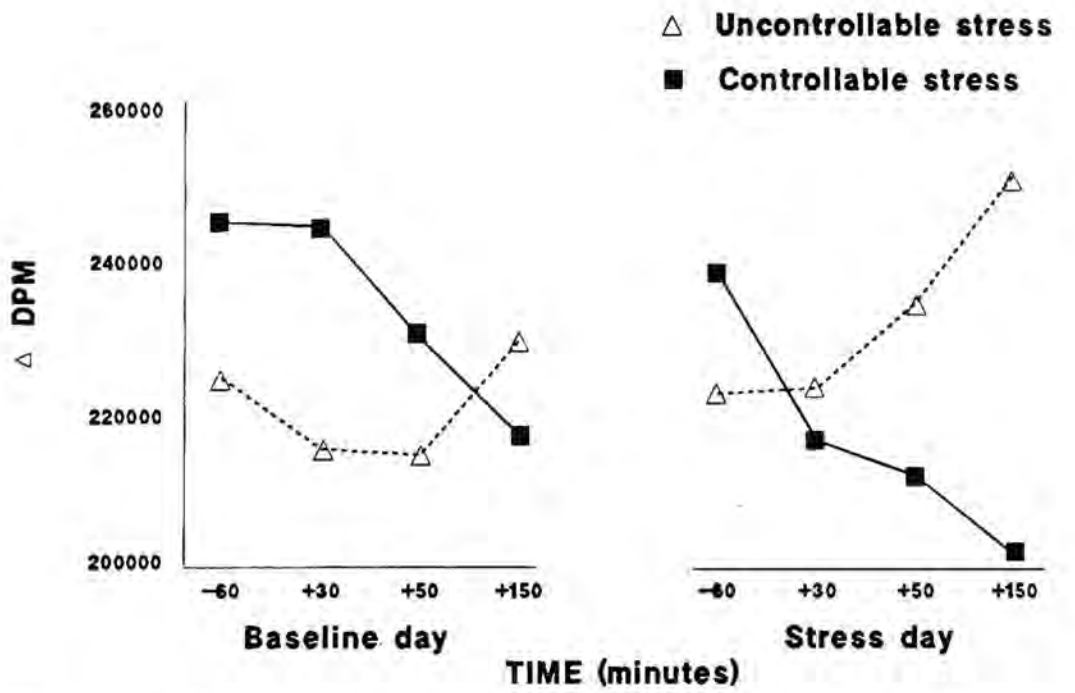

Figure 13. Lymphocyte proliferation to PHA (25 μg/ml) across baseline and stress sessions

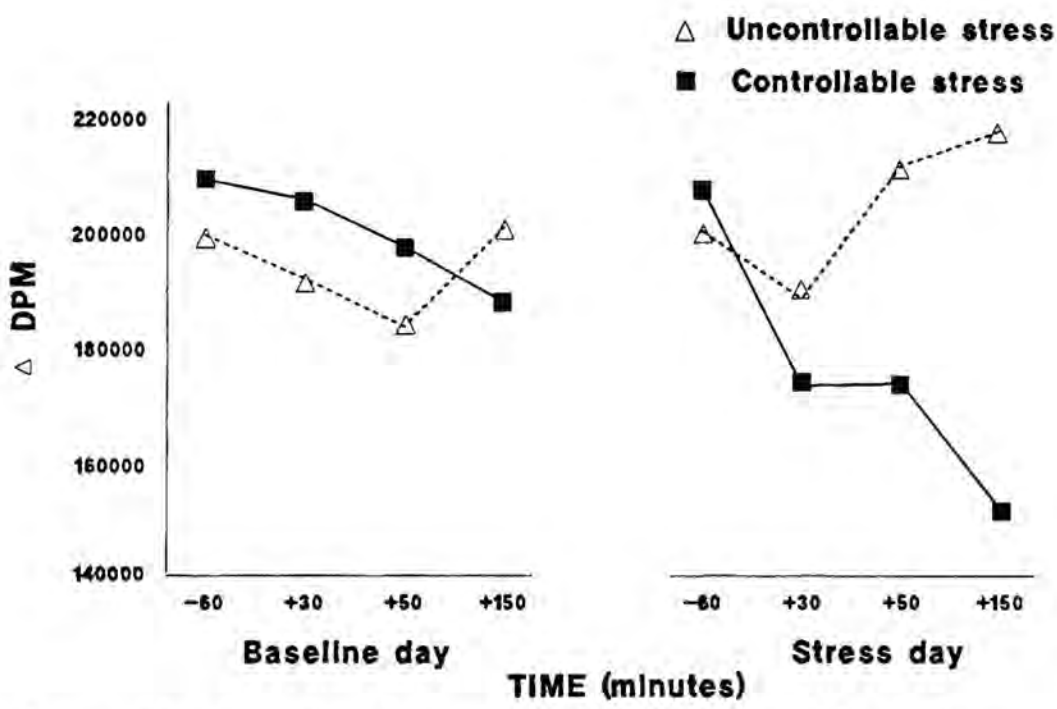

Figure 14. Lymphocyte proliferation to PHA (50 µg/ml) across baseline and stress sessions

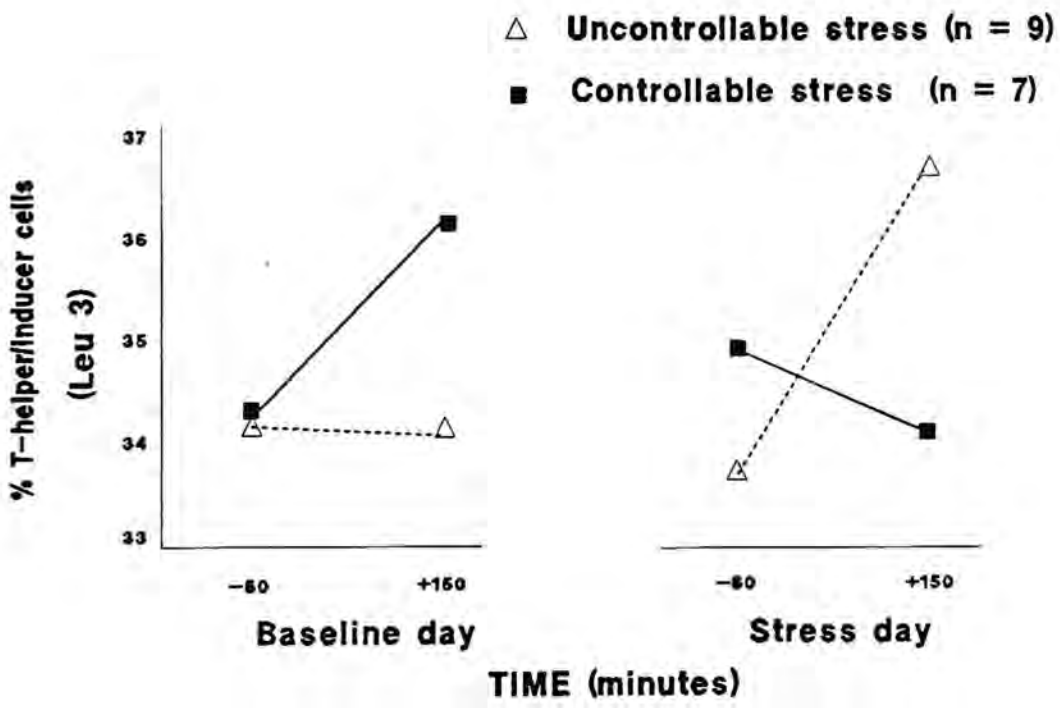

Figure 15. Percentages of T-helper cells across baseline and stress sessions

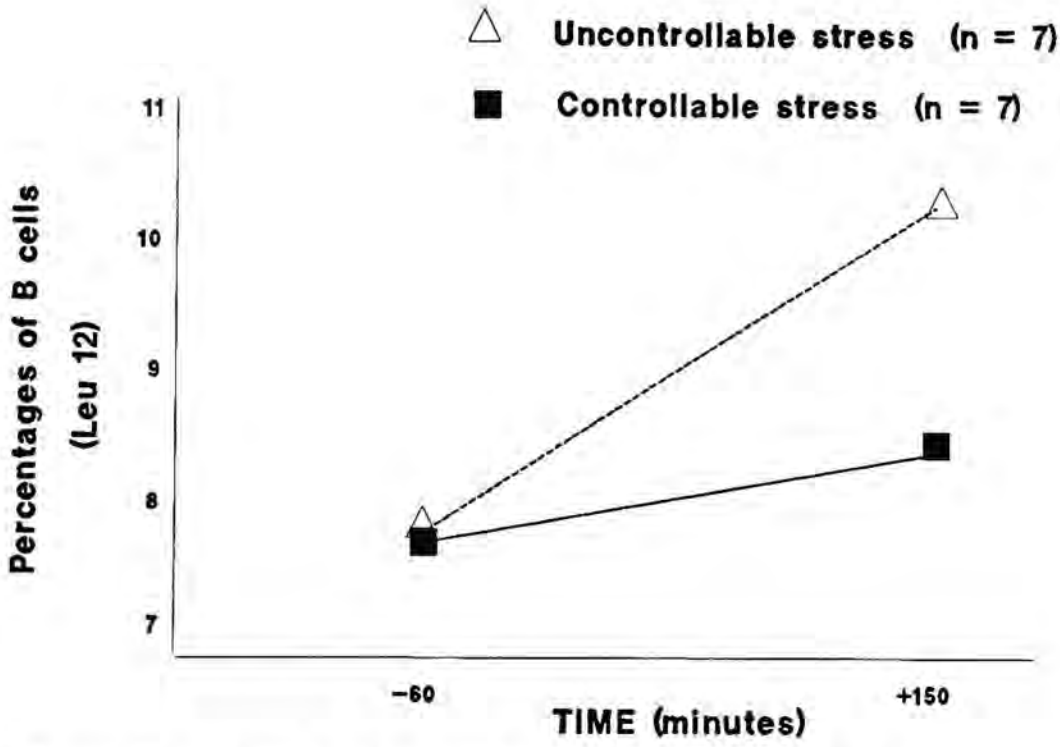

Figure 16. Group changes in percentages of B cells as a function of time

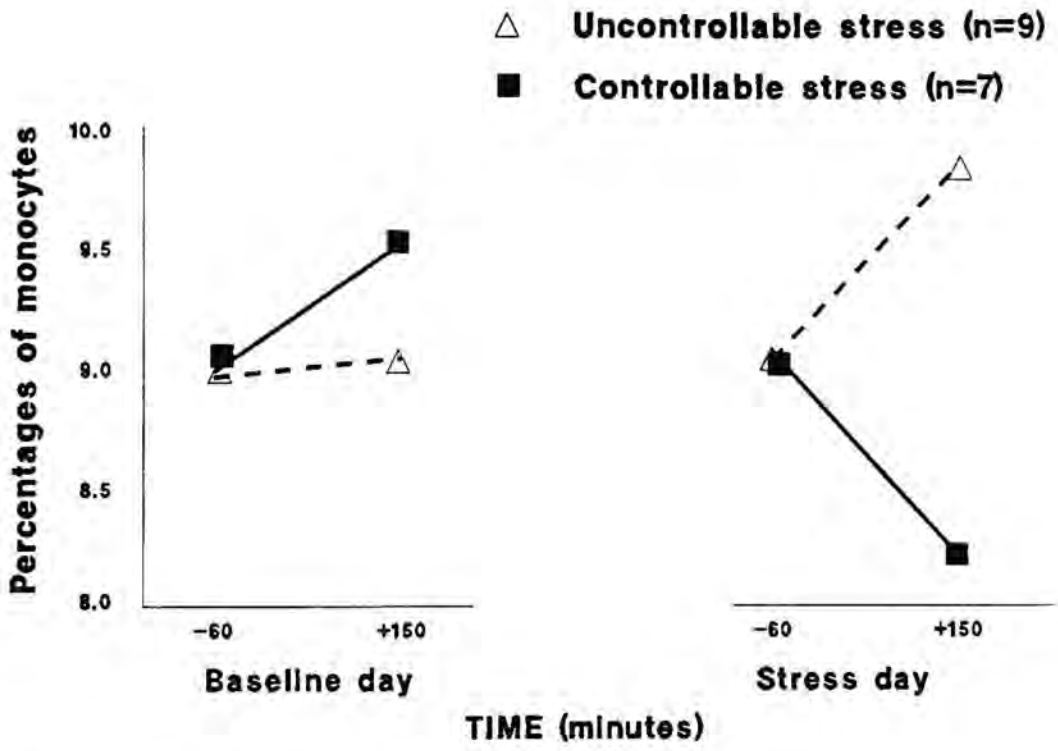

Figure 17. Percentages of monocytes across baseline and stress sessions

Appendix A: Description of Study Read to Potential Subjects, Telephone Screening Questions, and Consent Form

INFORMATION READ TO CALLERS INQUIRING ABOUT THE ADVERTISEMENT FOR RESEARCH PARTICIPANTS

STUDY : The effects of stress on task performance and on physiological processes

We are interested in why people do or do not get sick when they are under stress. To examine this question, we will look at how stressful stimuli affect, for example, the body's immune system, which is known to fight off disease. In addition, we are interested in how stress might affect learning. The study will be divided into two sessions, each lasting approximately three and one half hours. We would like to schedule you to come in on two days, and would like you to schedule your second visit no later than one week from your first visit. Because we are interested in how unexpected outside stimuli affect learning and biological processes, participants in this study will be exposed to stimuli consisting of noise and mild shock. These stimuli may be surprising and uncomfortable, but they will not be dangerous. We will be collecting several blood samples over the course of <u>each</u> session, and therefore will need to insert an I.V. (Have you ever had blood drawn from an I.V.?). In addition, you will be asked to fill out questionnaires and work on a performance tasks during each session. In order to be eligible for participation, you will need to first have a one hour physical (free) at NIH. If you are found to be completely healthy and can participate in both sessions of the study, you will be paid approximately $140.00, but you must complete both sessions of the study for payment.

TELEPHONE INTERVIEW

STUDY : The effects of stress on task performance and on physiological processes

ARE YOU CURRENTLY UNDER A DOCTORS SUPERVISION ? _____
IF YES, FOR WHAT REASON ? _____
_____

DO YOU HAVE ANY CURRENT OR CHRONIC MEDICAL PROBLEMS ? _____

HAVE YOU HAD A COLD OR THE FLU IN THE LAST TWO WEEKS ? _____

ARE ANY OF THE PEOPLE WITH WHOM YOU LIVE SICK WITH A COLD OR THE FLU ?
_____

HAVE YOU TAKEN ANY MEDICATIONS IN THE PAST SIX WEEKS ? _____

HAVE YOU EVER BEEN TREATED FOR (OR SEEN A DOCTOR) FOR PSYCHIATRIC ILLNESS ? _____

DO YOU USE ANY DRUGS AT ALL ? _____ DO YOU SMOKE ? _____
IF YES, HOW MUCH ? _____

HAVE YOU EVER HAD A HISTORY OF ALCOHOL ABUSE ? _____

HOW MUCH ALCOHOL DO YOU CONSUME REGULARLY IN A WEEK ? _____

HAVE YOU RECENTLY BEEN THROUGH A STRESSFUL EVENT (e.g., divorce or separation, death or serious illness of family member or close friend) ? _____

DO YOU READ AND WRITE ENGLISH ? _____

DO YOU HAVE ANY HEARING DEFECTS ? _____

HAVE YOU EVER PARTICIPATED IN A RESEARCH STUDY BEFORE ? _____
IF YES, WHERE WAS THE STUDY AND WHAT WAS IT ABOUT ? _____
_____

NAME : _____ AGE _____

DAYTIME PHONE # : _____ EVENING PHONE # : _____

DATES AND TIME SCHEDULED :
PHYSICAL _____
SESSION I _____
SESSION II _____

REMINDER CALL :
DATE MADE _____
BY WHOM _____

MEDICAL RECORD	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient

INSTITUTE: National Institute of Mental Health

STUDY NUMBER: 84-M-253 PRINCIPAL INVESTIGATOR: Steven M. Paul, M.D.

STUDY TITLE: The Behavioral, Psychophysiological and Neurobiological Assessment of Noise a Learning in Normal Subjects and Psychiatric Patients (Normal Controls and Euthymic Subjects)

INTRODUCTION

We invite you (or your child) to take part in a research study at the National Institutes of Health. It is important that you read and understand several general principles that apply to all who take part in our studies: (a) taking part in the study is entirely voluntary; (b) personal benefit may not result from taking part in the study, but knowledge may be gained that will benefit others; (c) you may withdraw from the study at any time without penalty or loss of any benefits to which you are otherwise entitled. The nature of the study, the risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. You are urged to discuss any questions you have about this study with the staff members who explain it to you.

PURPOSE

The purpose of the study is to find out whether attempting to avoid an unpleasant experience (loud noise and electric shock): (1) has significant effects on blood chemicals and skin electrical activity, (2) differs markedly in depressed and/or schizophrenic patients when compared to normal volunteers (thus showing a special vulnerability to such stress, (3) correlate with the absence or presence of symptoms in depressed and/or schizophrenic patients, and (4) influences later learning.

PROCEDURES

Before participating in the study, you will have a complete physical exam and psychiatric interview. If you are physically healthy and agree to participate you will be involved in two test days which will require 4 hours each day. For each test day, you will have a standard intravenous needle inserted in your arm for the purpose of drawing blood samples. The intravenous needle will stay in place for up to 4 hours. We will draw blood samples periodically throughout the 4-hour period and will take approximately 150 ml of blood each test day. Also your blood pressure and pulse will be monitored throughout the study and we will ask you to fill out rating scales of your emotional state at various times during the study.

About 1 hour after the intravenous line has been inserted, wires will be placed on your skin so that your skin's electrical activity can be measured. In brief, you will hear a loud noise through head phones and have electric shock administered to your arm from time-to-time and be given instructions regarding the termination of the noise and shock. After that you will be

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent to Participate In A Clinical Research Study NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study

STUDY NUMBER: _____ CONTINUATION: page 2 of 3 pages.

asked to complete a short learning test. After the learning test we will ask you to relax and read magazines for about 1 hour while a few more blood samples are collected. We will tell you everything, including your test scores, after you complete the study.

HAZARDS AND PRECAUTIONS

Although listening to loud noise and experiencing electric shock is safe, it may be moderately unpleasant and stressful for some subjects. For patients with schizophrenia and depression, it could make some of your symptoms temporarily worse. A psychiatrist will be present throughout the procedure to assist you if necessary. You may stop the procedure at any time. However, since stopping would compromise the study results, we would like you to continue if possible.

There is minor discomfort associated with insertion of the intravenous needle and having the needle in place for 4 hours. The total amount of blood drawn on each test day is approximately 150 ml which is less than one-half the amount taken during a standard blood donation.

REIMBURSEMENT

All normal volunteers and subjects with a past history of depression who are not in treatment at NIH now will be reimbursed in accordance with NIH guidelines.

MEDICAL RECORD	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient	continuation: page 3 of 3 pages

STUDY NUMBER: _____

OTHER PERTINENT INFORMATION

1. **Confidentiality.** When results of a study such as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records of Clinical Center patients are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

2. **Policy Regarding Research-Related Injuries.** The Clinical Center will provide short-term medical care for any physical injury resulting from your participation in research here. Neither the Clinical Center nor the Federal government will provide long-term medical care or financial compensation for such injuries, except as may be provided through whatever remedies are normally available under law.

3. **Payments.** If you are a patient, you are not paid for taking part in NIH studies. Exceptions for volunteers will be guided by Clinical Center policies.

4. **Problems or Questions.** Should any problem or question arise with regard to this study, with regard to your rights as a participant in clinical research, or with regard to any research-related injury, you should contact the principal investigator Steven M. Paul, M.D. or these other staff members also involved in this study: Carlos Pato, M.D. ; Robert Lirman, M.D. ; David Pickar, M.D. ;
Building 10, Room 4N212. Telephone: (301) 496-6295.
National Institutes of Health
Bethesda, Maryland 20205

5. **Consent Document.** It is suggested that you retain a copy of this document for your later reference and personal records.

COMPLETE APPROPRIATE ITEM BELOW, A or B:

A. **Adult Patient's Consent.**
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

_____
Signature of Adult Patient & Date Signed

B. **Parent's Permission for Minor Patient.**
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

_____
Signature of Parent(s) & Date Signed

_____
(if other than parent, specify relationship)

_____
Signature of Investigator & Date Signed

_____
Signature of Witness & Date Signed

PATIENT IDENTIFICATION

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY
• Adult Patient or • Parent, for Minor Patient

NIH-2514-1 (10-84) P.A. 09-25-0099

Appendix B: Background Questionnaire, Health Survey, and Blood Donor History Questionnaire

BACKGROUND QUESTIONNAIRE

1. Are you ? (circle one)

   Male
   Female

2. Your age _____

3. Your occupation _____

4. What is your marital status ?
   _____ Single
   _____ Married How long ? _____
   _____ Separated How long ? _____
   _____ Divorced How long ? _____
   _____ Widowed How long ? _____

5. If you were previously married, how long were you married ? _____

6. Number of family members living within 30 miles _____

7. Your highest educational level:
   ____ Grammar School
   ____ High School
   ____ Some College
   ____ College Degree
   ____ Graduate Work
   ____ Other (specify) _____

8. Number of people living at your residence ? _____

9. Type of residence:
   _____ Apartment
   _____ Single family home
   _____ Two family home
   _____ Three family home
   _____ Townhouse
   _____ Other (specify) _____

10. Do you own or rent ? _____

11. List your primary reasons for selecting this place to live (e.g., close to schools, close to work, etc._____
_____
_____

12. Approximate annual income:
   _____ Under $10,000/year
   _____ $10,000 - $15,000/year
   _____ $15,001 - $20,000/year
   _____ $20,001 - $30,000/year
   _____ $30,001 - $40,000/year
   _____ $40,001 - $50,000/year
   _____ over $50,000/year

HEALTH QUESTIONNAIRE

The following questionnaire consists of a number of items dealing with your health and related behavior. Some of these items address current behaviors while other questions deal with changes in your behavior over the past few years. The items on this questionnaire are similar to those on other forms you have completed for us. Nevertheless, for us to be able to make the most meaningful evaluation of your community, it is important for you to answer each of the items as accurately and honestly as possible. However, if for any reason there are questions which you would prefer not to answer, go ahead and skip those particular questions.

Age_____ Height_____ Weight_____

1. In general, how would you describe your health right now?
   _____Very poor
   _____Poor
   _____Fair
   _____Good
   _____Excellent

2. Do you have any permanent or chronic health problems or any problems which have lasted 3 months or more? Y_____ N_____ If yes, please specify:_____

3. Has your health changed in the last 6 months? Did it improve, remain about the same, or become worse?
   _____Improve
   _____Remain the same
   _____Become worse

4. Have you visited your doctor in the past 12 months? Y_____ N_____
   If yes, for what reason(s) and how many times?_____

5. Have you sought medical attention for yourself by telephone during the past 12 months? Y_____ N_____
   If yes, for what reason(s) and how many times? _____

6. Have you been hospitalized for any reason in the last 3 years?
   Y_____ N_____
   If yes, please give the reasons, dates, and lengths of stay.

Date	Reason for Hospitalization	Length of Stay
_____	_____	_____
_____	_____	_____
_____	_____	_____

YOUR RECENT HEALTH
(MODIFIED CORNELL MEDICAL INDEX)

Please complete this health questionnaire for the past two years.
Circle "Y" for "Yes"; circle "N" for "No"

A	1987	1988
Do you need glasses to read?	Y / N	Y / N
Do you need glasses to see things at a distance?	Y / N	Y / N
Are your eyes often red or inflammed?	Y / N	Y / N
Do you have constant noises in your ears?	Y / N	Y / N
B		
Do you have to clear your throat frequently?	Y / N	Y / N
Is your nose constantly stuffed up?	Y / N	Y / N
Do you often catch severe colds?	Y / N	Y / N
Do you get hay fever?	Y / N	Y / N
Do you suffer from asthma?	Y / N	Y / N
Have you ever had a chronic chest condition?	Y / N	Y / N
C		
Has a doctor ever said your blood pressure was too high?	Y / N	Y / N
Has a doctor ever said your blood pressure was too low?	Y / N	Y / N
Are you often bothered by thumping of the heart?	Y / N	Y / N
Does you heart often race like mad?	Y / N	Y / N
Has a doctor ever said you had heart trouble?	Y / N	Y / N
Does heart trouble run in your family?	Y / N	Y / N
D		
Have you lost more than half your teeth?	Y / N	Y / N
Are you troubled by bleeding gums?	Y / N	Y / N
Is your appetite always poor?	Y / N	Y / N
Do you often suffer from an upset stomach?	Y / N	Y / N
Are you often sick to your stomach?	Y / N	Y / N
Do you suffer from indigestion?	Y / N	Y / N
Has a doctor ever said you have stomach ulcers?	Y / N	Y / N
Do you suffer from constant stomach trouble?	Y / N	Y / N
Have you ever had severe bloody diarrhea?	Y / N	Y / N
Do you constantly suffer from bad constipation?	Y / N	Y / N
Have you ever had rectal hemorrhoids?	Y / N	Y / N
Have you ever had jaundice (yellow eyes and skin)?	Y / N	Y / N
Have you ever had serious liver or gall bladder trouble?	Y / N	Y / N
E		
Are your joints often painfully swollen?	Y / N	Y / N
Do your muscles and joints constantly feel stiff?	Y / N	Y / N
Are you crippled with severe rheumatism (arthritis)?	Y / N	Y / N
Do pains in the back make it hard for you to keep up with your work?	Y / N	Y / N

F	1987	1988
Is your skin very tender?	Y / N	Y / N
Do you sweat a great deal even in cold weather?	Y / N	Y / N
Are you often bothered by severe itching?	Y / N	Y / N
Does your skin often break out in a rash?	Y / N	Y / N
G		
Do you suffer badly from frequent severe headaches?	Y / N	Y / N
Are headaches common in your family?	Y / N	Y / N
Do you often have spells of severe dizziness?	Y / N	Y / N
Do you have constant numbness or tingling in any part of your body?	Y / N	Y / N
Have you at times had a twitching of the face, head or shoulders?	Y / N	Y / N
Did you ever have a fit or convulsion (epilepsy)?	Y / N	Y / N
Do you bite your nails badly?	Y / N	Y / N
Are you troubled by stuttering or stammering?	Y / N	Y / N
H		
Have you ever had treatment for your genitals?	Y / N	Y / N
Has a doctor ever said you had a hernia?	Y / N	Y / N
Has a doctor ever said you had kidney or bladder disease?	Y / N	Y / N
I		
Do you often get spells of complete exhaustion or fatigue?	Y / N	Y / N
Does working tire you out completely?	Y / N	Y / N
Do you usually get up tired and exhausted in the morning?	Y / N	Y / N
J		
Are you frequently ill?	Y / N	Y / N
Are you frequently confined to bed by illness?	Y / N	Y / N
K		
Do you have diabetes?	Y / N	Y / N
Did a doctor ever say you had a goiter (in your neck)?	Y / N	Y / N
Did a doctor ever treat you for a tumor or cancer?	Y / N	Y / N
Do you suffer from any chronic disease?	Y / N	Y / N
Are you definitely underweight?	Y / N	Y / N
Are you definitely overweight?	Y / N	Y / N
Did you ever have a serious operation?	Y / N	Y / N
Did you ever have a serious injury?	Y / N	Y / N
Do you often have small accidents or injuries?	Y / N	Y / N

L	1987	1988
Do you usually have great difficulty falling asleep or staying asleep?	Y / N	Y / N
Do you find it impossible to take a regular rest period each day?	Y / N	Y / N
Do you smoke more than 20 cigarettes each day?	Y / N	Y / N
Do you drink more than six cups of coffee or tea each day?	Y / N	Y / N
Do you usually take two or more alcoholic drinks a day?	Y / N	Y / N
M		
Do you sweat or tremble a lot during examination or questioning?	Y / N	Y / N
Do you get nervous and shaky when approached by a superior?	Y / N	Y / N
Does your thinking get completely mixed up when you have to do things quickly?	Y / N	Y / N
Must you do things very slowly in order to do them without mistakes?	Y / N	Y / N
N		
Do you feel alone and sad at a party?	Y / N	Y / N
Do you usually feel unhappy and depressed?	Y / N	Y / N
Do you often cry?	Y / N	Y / N
Are you always miserable and blue?	Y / N	Y / N
Does life look entirely hopeless?	Y / N	Y / N
Do you often wish you were dead and away from it all?	Y / N	Y / N
O		
Does worrying continually get you down?	Y / N	Y / N
Does every little thing get on your nerves and wear you out?	Y / N	Y / N
Are you considered a nervous person?	Y / N	Y / N
Did you ever have a nervous breakdown?	Y / N	Y / N
Did anyone in your family ever have a nervous breakdown?	Y / N	Y / N
Were you ever a patient in a mental hospital?	Y / N	Y / N
Was anyone in your family ever in a mental hospital?	Y / N	Y / N
P		
Are you extremely shy or sensitive?	Y / N	Y / N
Are your feelings easily hurt?	Y / N	Y / N
Does criticism always upset you?	Y / N	Y / N
Are you considered a touchy person?	Y / N	Y / N
Do people usually misunderstand you?	Y / N	Y / N

Q	1987	1988
Do you have to be on your guard even with friends?	Y / N	Y / N
Do you always do things on sudden impulse?	Y / N	Y / N
Are you easily upset or irritated?	Y / N	Y / N
Do you go to pieces if you don't constantly control yourself?	Y / N	Y / N
Do little annoyances get on your nerves and make you angry?	Y / N	Y / N
Does it make you angry to have anyone tell you what to do?	Y / N	Y / N
Do people often annoy and irritate you?	Y / N	Y / N
Do you flare up in anger if you can't have what you want right away?	Y / N	Y / N
Do you often get into a violent rage?	Y / N	Y / N

R		
Do you often shake or tremble?	Y / N	Y / N
Are you constantly keyed up and jittery?	Y / N	Y / N
Do sudden noises make you jump or shake badly?	Y / N	Y / N
Do you tremble or feel weak whenever someone shouts at you?	Y / N	Y / N
Do you become scared at sudden movements or noises at night?	Y / N	Y / N
Do frightening thoughts keep coming back in your mind?	Y / N	Y / N
Do you often become suddenly scared for no good reason?	Y / N	Y / N
Do you often break out in a cold sweat?	Y / N	Y / N

12. Do you take any prescsription drugs? Y_____ N_____
If yes, specify what drugs, for what health problems, how much, and how often: _____

13. Do you take any non-prescription drugs? Y_____ N_____
If yes, please specify which types, for what health problems, how much, and how often.
_____aspirin_____
_____vitamin supplements_____
_____mineral supplements_____
_____allergy medications_____
_____sleeping pills_____
_____other (specify)_____

14. Do you drink beer or wine? Y_____ N_____
If yes, how many beers or glasses of wine do you drink each week?
_____1-5
_____6-10
_____11-15
_____over 15

15. Do you drink alcoholic drinks other than beer or wine?
Y_____N_____
If yes, how many drinks do you have each week?
_____1-5
_____6-10
_____11-15
_____over 15

16. How often do you have two or more alcoholic drinks in one day?
_____never
_____rarely
_____1-2 times per week
_____3-4 times per week
_____5-6 times per week
_____every day

17. Do you smoke? Y_____ N_____
If yes, how many cigarettes do you smoke each day?
_____under 10 (½ pack)
_____10-20 (½-1 pack)
_____20-40 (1-2 packs)
_____over 40 (2 packs)
How often do you smoke cigars or pipe tobacco?
_____never
_____occasionally
_____once per day
_____several times per day
_____many times per day
How often do you smoke more than one pack of cigarettes in one day?
_____never
_____occasionally
_____often
_____always

18. List any idiosyncratic tendencies you have (e.g., biting fingernails, cracking knuckles, tapping of foot while sitting, etc.):_____

19. How many hours of sleep do you get each night?
_____under 6
_____6-7½
_____about 8
_____8½-10
_____over 10

20. Have you noticed a change in your sleeping habits in the past 3 years (other than job/school related events)? Y_____ N_____
If yes, in what period of time did you notice a change?
_____0-6 mos. ago
_____7-12 mos. ago
_____1-2 yrs. ago
_____2-3 mos. ago

21. Please indicate the number of 8 ounce servings of each of the following beverages you consume daily?
    coffee_____
    tea_____
    soft drinks_____
    milk_____
    water_____
    other_____

22. How often do you urinate each day?
    _____1-2 times
    _____3-4 times
    _____5-6 times
    _____7-8 times
    _____9 or more times

23. Are you on a diet? Y_____ N_____
    If yes, what kind?
    _____fasting (specify number of days at a time_____)
    _____calorie counting (specify number of calories per day_____)
    _____carbohydrate counting (specify number per day_____)
    _____other (specify_____)

24. What was your weight 6 months ago?_____ 1 year ago?_____
    2 years ago?_____ 3 years ago?_____

25. How often do you exercise?
    _____every day
    _____5-6 times per week
    _____3-4 times per week
    _____1-2 times per week

26. What kind of exercise do you do?_____
    _____
    _____

BLOOD DRAWING HISTORY

PLEASE READ AND ANSWER THE FOLLOWING QUESTIONS :

1. **NORMALLY, DOES THE THOUGHT OF HAVING YOU BLOOD DRAWN MAKE YOU UNCOMFORTABLE ?**

1	2	3	4	5	6	7
not at all						extremely

2. **DOES THE THOUGHT OF NEEDLES IN GENERAL DISTURB YOU ?**

1	2	3	4	5	6	7
not at all						extremely

3. **HAVE YOU EVER DONATED BLOOD BEFORE ?** YES NO

IF YES, ABOUT HOW MANY TIMES ? _____

HOW DID YOU FIND THE OVERALL BLOOD DONATING EXPERIENCE ?

1	2	3	4	5	6	7
extremely unpleasant						extremely pleasant

4. **IF YOU HAVE NEVER DONATED BLOOD BEFORE, WHICH ONE OF THE FOLLOWING REASONS BEST DESCRIBE WHY ?**

____ **the time involved**
____ **the unpleasantness of the procedure**
____ **concern about communicating disease**
____ **health reasons**
____ **personal reasons**

Appendix C: Stress Analog Scale and Noise/Shock Questionnaire

ANALOG SCALE NO. 2

SUBJECT NO.: _____ DATE : _____ TIME POINT : _____

INSTRUCTIONS : Below are 10 words which describe the feelings people have. Please read each one carefully and rate how much you have had that feeling during the past 10 minutes, including now. You may mark anywhere on each line.

	NOT AT ALL		MODERATELY	EXTREMELY
1. TENSE	|_____	_____	_____	_____|
2. HAPPY	|_____	_____	_____	_____|
3. FRUSTRATED	|_____	_____	_____	_____|
6. RESTLESS	|_____	_____	_____	_____|
5. CALM	|_____	_____	_____	_____|
6. SAD	|_____	_____	_____	_____|
7. STRESSED	|_____	_____	_____	_____|
8. ANGRY	|_____	_____	_____	_____|
9. DOWN IN THE DUMPS	|_____	_____	_____	_____|
10. CLEAR THINKING	|_____	_____	_____	_____|

NOISE AND SHOCK QUESTIONNAIRE

SUBJECT NO. : _____ DATE : _____

INSTRUCTIONS : Below are questions regarding the experiment. Please read each one carefully and answer the questions by placing a mark anywhere on each corresponding line.

1. HOW LOUD DID YOU THINK THE NOISE WAS?

NOT AT ALL MODERATELY EXTREMELY

|_____|_____|_____|_____|

2. HOW SUCCESSFUL DO YOU FEEL YOU WERE IN THE NOISE-SHOCK CONDITION ?

NOT AT ALL MODERATELY EXTREMELY

|_____|_____|_____|_____|

3. HOW HELPLESS DID YOU FEEL DURING THE NOISE-SHOCK CONDITION ?

NOT AT ALL MODERATELY EXTREMELY

|_____|_____|_____|_____|

4. HOW STRONG DID YOU THINK THE SHOCK WAS ?

NOT AT ALL MODERATELY EXTREMELY

|_____|_____|_____|_____|

5. HOW MUCH CONTROL DID YOU HAVE OVER STOPPING THE NOISE AND SHOCK ?

NOT AT ALL MODERATELY EXTREMELY

|_____|_____|_____|_____|

6. WAS THE NOISE-SHOCK CONDITION BORING ?

NOT AT ALL MODERATELY EXTREMELY

|_____|_____|_____|_____|

7. HOW UNPLEASANT WAS THE NOISE-SHOCK CONDITION ?

NOT AT ALL MODERATELY EXTREMELY

|_____|_____|_____|_____|

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